Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 45. DECEMBER, 1940. NO. 3.

SECTION MEETINGS

CLEVELAND	
Western Reserve University	December 13, 1940
ILLINOIS	
University of Chicago	December 10, 1940
MINNESOTA	
University of Minnesota	November 20, 1940
PACIFIC COAST	
Stanford Lane Hospital	December 4, 1940
WESTERN NEW YORK	
N. Y. State Agricultural Exp. Station	October 19, 1940
University of Rochester	December 14, 1940

11824

Influence of Protamine Zinc Insulin upon Appetite During Anorexia of Vitamin B_1 Deficiency.

RICHARD H. BARNES AND EATON M. MACKAY.

From The Scripps Metabolic Clinic, La Jolla, California.

Protamine zinc insulin has a remarkable effect upon the appetite of normal albino rats[1, 2] leading to a marked hyperalimentation and the deposition of an excess of body fat. The influence of this insulin preparation upon the decreased appetite in the anorexia due to vitamin B_1 deficiency[3] was examined with the idea of throwing

---

[1] MacKay, E. M., and Callaway, J. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 406.

[2] MacKay, E. M., Callaway, J. W., Barnes, R. H., *J. Nutri.*, 1940, **20**, 59.

[3] Williams, R. R., and Spies, T. D., *Vitamin B_1*, The Macmillan Company, New York, 1938, pp. 288-290.

more light on the mechanism of the insulin hyperalimentation in normal animals,[2] of gaining information relative to the reason for appetite failure when this vitamin is deficient and the question of the necessity of vitamin B_1 for the conversion of carbohydrate to fat.[4, 5, 6]

Four groups of six 60-day-old male rats were placed upon a diet* composed of vitamin-free casein 20, sucrose 71, standard (Osborne and Mendel) salt mixture 4 and dried and powdered autoclaved brewer's yeast free of vitamin B_1 5 parts. In addition there were 10 mg vitamin B_6, 10 mg riboflavin and 20 mg nicotinic acid per 100 g of food. This diet was fed to groups A and B. Groups C and D were given the same diet with the addition of .05% thiamin chloride (Vit. B_1). Every second day each of the rats in all groups were given one drop of a fish liver oil containing 60,000 U.S.P. Vit. A units and 8500 U.S.P. Vit. D units per gram.

Figure 1 presents our experimental results. All of the groups gained well for some 12 days after which those (A and B) without the B_1 supplement commenced to lose weight. When the food intake was measured after 20 days the poor appetite of the B_1-deficient rats was very obvious. At this time the administration of protamine zinc insulin (Squibb's product in a strength of 40 u. per cc) was begun. Group D on an adequate diet were given 2 units subcutaneously morning and evening. Their food intake rapidly increased and their weight curve was obviously superior to their controls (Group C) on an adequate diet. A vitamin-deficient group which is not charted in Fig. 1 but which had a weight curve about like Group A were given the 2-unit insulin dose twice daily beginning at 81 days of age and all 6 rats in the group died in hypoglycemia within 3 days. Their appetite was not increased. The B_1-deficient group B were therefore given 1 unit of insulin each twice daily. There was again no increased food intake and 3 of the 6 rats died in hypoglycemia before the end of the 8-day period (89 days old) when vitamin B_1 administration was commenced. At this time the rats in both groups A and B were given subcutaneously 5 mg thiamin chloride in aqueous solution every day. The food intake and body weight of both groups increased at once. The insulin-treated group did not do much better than the others until the dose of insulin was increased to 2 units twice daily when they were 93 days old.

---

[4] Whipple, D. V., and Church, D. F., *J. Biol. Chem.*, 1936, **114**, cvii.

[5] Whipple, D. V., and Church, D. F., *J. Biol. Chem.*, 1937, **119**, cii.

[6] McHenry, E. W., and Gavin, G., *J. Biol. Chem.*, 1938, **125**, 653.

* Their previous diet contained barely sufficient thiamin chloride for normal growth.

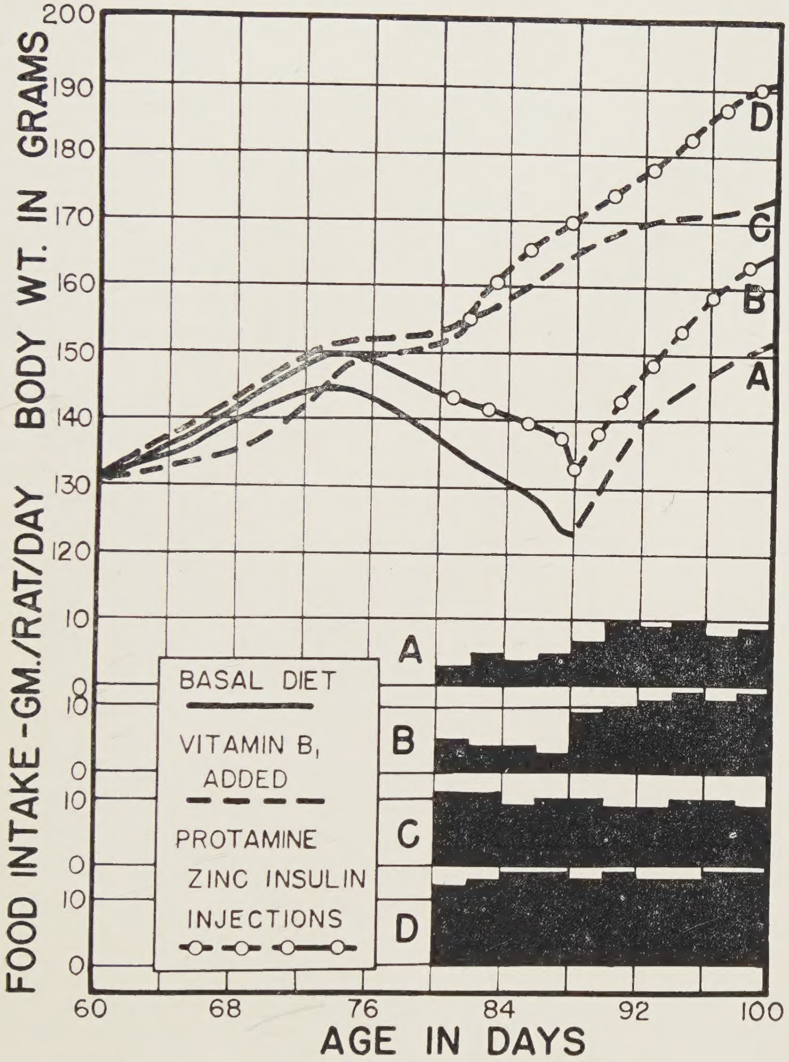


FIG. 1.

It is quite obvious from our experiments that vitamin B_1 in adequate amounts must be present in the organism if protamine zinc insulin is to lead to an increase in appetite. In normal rats we inclined toward the idea that the insulin hyperalimentation was a result of the depression of the blood sugar level.[2] Vitamin B_1-deficient rats, however, died in hypoglycemia without any increase in the food intake whatever. Our diet was devised to study the conversion of carbohydrate to fat and so is practically fat-free.

Rats suffering from vitamin B_1 deficiency have an aversion to both carbohydrate and protein[7] and it is possible that insulin might influence the appetite for fat in B_1 deficiency but we have found the fat appetite less affected than that for carbohydrate in normal rats.

Summary. The administration of protamine zinc insulin which leads to a marked hyperalimentation in normal rats is without effect upon the depressed appetite of rats suffering from vitamin B_1 deficiency. These deficient rats succumb in hypoglycemia without an increase in their food intake when the insulin is administered. When thiamin chloride is given to B_1-deficient rats their appetite responds not only to the vitamin therapy but to protamine zinc insulin as do normal animals.

11825 P

Secretion of Radio-Strontium in Milk of Two Cows Following Intravenous Administration.*

L. A. ERF,† AND CHARLES PECHER.‡ (Introduced by J. H. Lawrence.)

From Crocker Radiation Laboratory, University of California, Berkeley, California.

The composition of milk of cows varies markedly and is quickly influenced by the food ingested. To the pediatrician interested in infant nutrition this fact carries great significance. To determine, ultimately, the percentage of ingested calcium that is normally secreted in the milk of cows, the following preliminary experiment was made.

Method. Harold Walke, *et al.*,[1] have shown that the only radiocalcium isotope suitable for biological investigation is ${}_{20}Ca^{45}$. However, the poor yield of this isotope and the softness of its emitted radiations prohibit, at the present time, its use as a tracer in large

---

[7] Richter, C. P., Holt, L. E., Barelare, B., Jr., and Hawkes, C. D., *Am. J. Physiol.*, 1938, **124**, 596.

* We wish to thank the crew managing the Berkeley cyclotron for producing the ${}_{38}Sr^{89}$ used, and Dr. O. W. Schalm of the Department of Veterinary Science, University of California, who secured for us the cows used. The investigation reported here was aided by a grant from the John and Mary R. Markle Foundation for Medical Research.

† Wm. R. Kenan, Jr., Research Fellow.

‡ Fellow the Belgian American Education Foundation.

[1] Walke, H., Thompson, F. C., and Holt, G., *Phys. Rev.*, 1940, **57**, 177.

animals. Recently Pecher[2] has demonstrated a marked similarity in the metabolism of radio-calcium and radio-strontium of mice and rats. This suggested the use of radio-strontium instead of radio-calcium in determining the rate at which alkaline earth ions in the plasma are secreted into milk, since $_{38}Sr^{89}$ has a half life of 55 days and emits pure beta-particles at energies averaging about 750,000 electron volts. At present there is no significant evidence that living cells can distinguish an element from its radioactive isotope. To avoid the differences in the rates of absorption from the intestines between calcium and strontium, 50 cc of an isotonic solution of a mixture of inert and radioactive strontium lactate (850 mg of strontium) which emitted 11.2 microcuries of beta radiation per cc were given intravenously to each of 2 Holstein cows both in the fourth month of their lactation period and both producing about the same quantity of milk. The milk was obtained hourly the first 6 hours after administration and twice a day for the following 4 days.

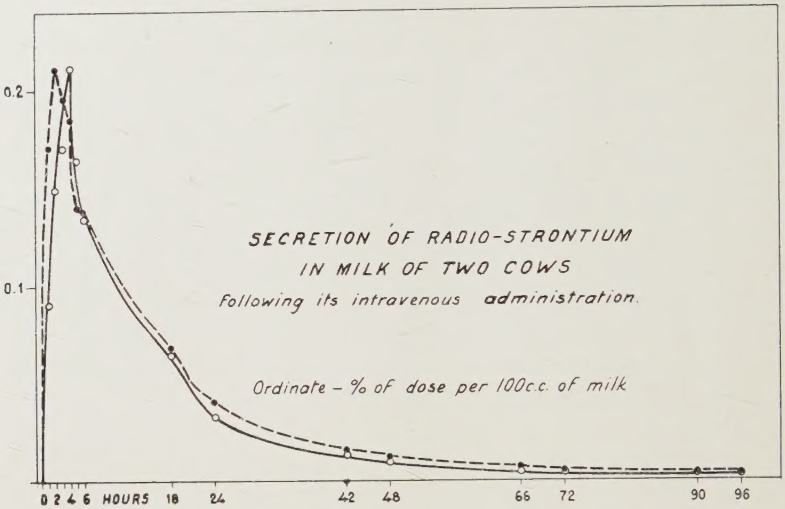
Ten cc aliquots of each of the 28 samples were ashed at 400°C. 100 cc portions of the second, fourth, seventh and ninth samples from each cow were placed in 400 cc of ethyl alcohol and agitated at irregular intervals for 10 days. The samples were filtered and the filtrates and precipitates ashed separately. 50 cc of the fifth sample of one cow was placed in a thick collodion bag on which 2 meters of water pressure was constantly applied for 72 hours. The ultrafiltrate and the residue were ashed separately. The various ashed samples were assayed for radioactivity on a standardized DuBridge electrometer and compared with aliquots of the dose of radio-strontium administered.

CHART I.

	Period after i.v. administration, hr	No. ccs of milk secreted	% of dose of $_{38}Sr^{89}$ present in milk
Cow 227	0-6	2329	3.9
	6-30	8700	4.9
	30-54	8000	1.24
	54-78	7600	.54
	78-102	7700	.42
	Total	34355	11.00
Cow 269	0-6	1435	2.3
	6-30	6800	3.85
	30-54	7400	1.11
	54-78	6100	.43
	78-102	5400	.19
	Total	27135	7.88

---

[2] Pecher, Chas., Proc. Soc. Exp. Biol. and Med., to be published.



Results. The accompanying graph and chart reveal that 11% of the dose of radio-strontium administered to the first cow was found in the 34,355 cc of milk secreted during the first 4¼ days, and 7.88% in the 27,135 cc of milk secreted by the second cow during the same period. The percentages of the dose found per 100 cc of milk from the two cows were almost identical (graph). The greatest amounts of radio-strontium were found in the milk during the first few hours following administration. This suggests that much of the calcium found in milk may be derived from that present in the diet, and Pecher has shown, in mice, that some is derived from that most recently deposited in the trabecular portion of bones. 97-99% of $_{38}Sr^{89}$ in all the samples studied was present in the precipitate of the alcohol-milk mixture and only 16% passed through the collodion bag as an ultra-filtrate. It is impossible to separate the calcium phosphate-calcium caseinate complex of milk and from the above findings it is clear that it is essentially impossible to separate the strontium complex also.

Summary. 7.88% and 11% of a dose of radio-strontium were secreted in the milk of two Holstein cows during 4¼ days following its intravenous administration.

11826 P

Determination of Vitamin B_1 Requirement of Infants by Means of Urinary Excretion of Thiamin.

Elizabeth M. Knott. (Introduced by Frederic W. Schlutz.)

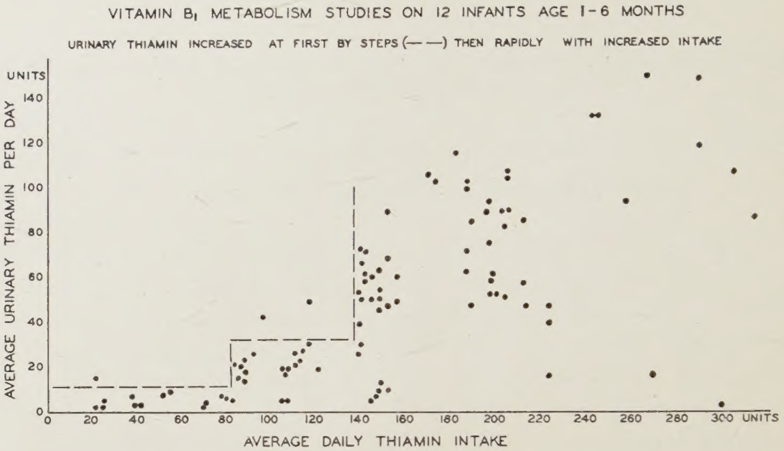
From the Department of Pediatrics, University of Chicago.*

In order to determine the level of vitamin B_1 intake which will give optimum conditions for development for the young infant, a series of 5-day balance studies have been conducted on each of 12 healthy male infants during the first 6 months of their lives. Thus far a total of 98 metabolism periods have been completed. The vitamin B_1 intake of the infants has been controlled either through the amount and type of milk fed each day, or by the additions of pure thiamin to the formulas. The range of total intake has been from 21 to 313 units of thiamin per day. Periodically for each infant, quantitative collections of urine and feces have been made for 5 consecutive days. These materials, as well as the various milks, have been assayed biologically for their thiamin content.

It was apparent early in the study that infants had absorbed thiamin from milk formulas very completely since only small quantities of the vitamin were excreted in the stools and in general the amounts of thiamin excreted in the urine increased with increases in intake. In the accompanying chart the amounts of urinary thiamin have been plotted in relation to the intake. A study of these plotted results reveals 2 shifts in the pattern of urinary excretion which seem significant. Below 80 units of intake per day a constantly low quantity of thiamin was excreted in the urine. At 140 units or more of intake, the thiamin appeared to be excreted in much larger amounts as higher levels of the vitamin were fed. The average urinary thiamin for successive increments of 20 units of intake were as follows: 6, 6, 4—19, 22, 34—47, 104, 77, 76, 34, 118, 83, 90, 96. The 2 bars are placed in the sequence at 80 and 140 units of intake per day. These data seem to indicate that 80 units may be the minimum requirement, and 140 units the maximum amount needed for this age infant. Perhaps the range from 100 to 150 units of thiamin may be considered the optimum intake for a young healthy infant, and that above this level of ingestion excess vitamin is excreted rapidly in the urine. The optimum for the baby, however, cannot be determined by economy of vitamin alone, and infants may require a considerable continuous wastage.

---

* Assistance in the preparation of these materials was furnished by the personnel of Works Project Administration Official Project No. 30230.


VITAMIN B₁ METABOLISM STUDIES ON 12 INFANTS AGE 1-6 MONTHS

URINARY THIAMIN INCREASED AT FIRST BY STEPS (— —) THEN RAPIDLY WITH INCREASED INTAKE

EACH DOT IS THE AVERAGE FOR ONE INFANT DETERMINED DURING A 5-DAY METABOLISM PERIOD

In order to determine thiamin requirements more exactly we have also studied the amounts of cocarboxylase found in the blood of young infants. Preliminary results show that at an intake increasing from 42 to 56 units of thiamin per day during 9 weeks of study, one infant was not able to maintain the level of his blood vitamin, whereas a second infant did maintain the level of cocarboxylase in his blood when given an intake ranging from 49 to 58 units of thiamin per day over the same period. This second infant, however, was the best infant we have ever studied so far as utilization of nutrients is concerned, and it is probable that the minimum requirement for the average infant is above the level of 60 units of thiamin per day which these infants received. These blood data tend to substantiate the conclusion based on urinary excretion that 80 units of intake represent the minimum requirement. Further investigations are now under way to determine the lowest thiamin intake which is compatible with health, and also the optimal amounts of vitamin B_1 for the best development of the infant.

11827 P

Buccal Absorption of α-Estradiol in Propylene Glycol.

UDALL J. SALMON AND SAMUEL H. GEIST.

From the Mt. Sinai Hospital, New York.

In the treatment of estrogen deficiency states, it is frequently necessary to administer estrogens for prolonged periods of time.

The usual method of administration consists of subcutaneous or intramuscular injections. It would be highly advantageous if one could administer estrogens in effective amounts without submitting the patient to the annoyance of repeated injections. Although it has been shown that estrogens are absorbed through the skin and the intestinal tract, neither method is satisfactory for therapeutic purposes.

In the present communication, we wish to describe a method for administering estrogens which is both simple and effective. It consists of sublingual administration of α-estradiol in a propylene glycol solution.* The solution contains 0.5 mg of α-estradiol per cc of propylene glycol. Four drops are placed under the tongue and the patient is instructed not to swallow for five minutes. This can be done several times a day.

The present study was performed on a series of 8 women with

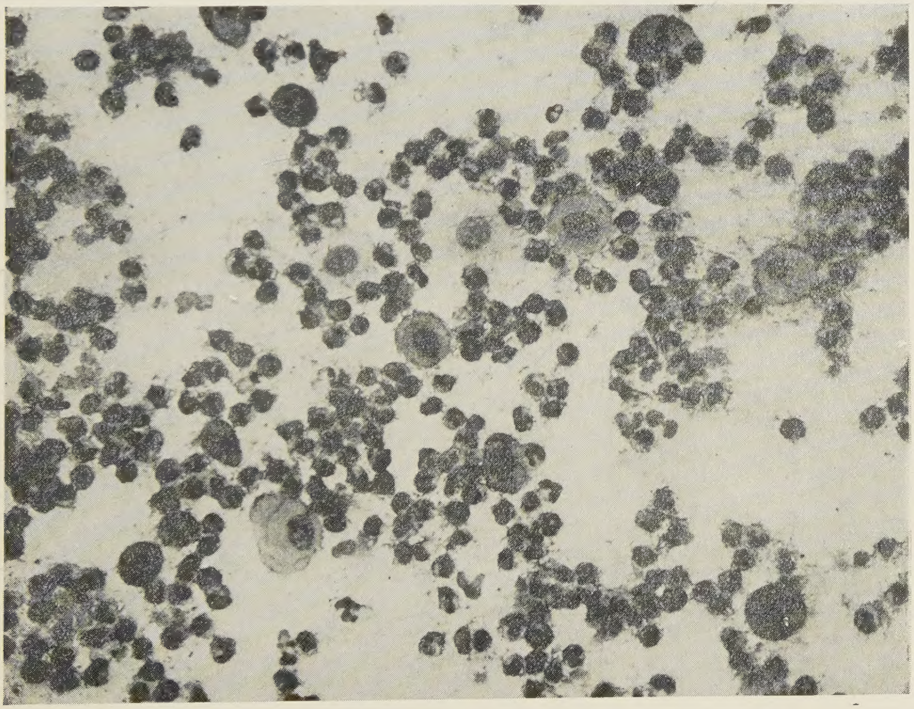


FIG. 1.

Patient S.B., age 54, menopause 6 years ago. Pre-treatment vaginal smear revealing marked degree of estrogen deficiency.

---

* For the materials used in this investigation, we are indebted to Dr. Edward Henderson, of the Schering Corporation, Bloomfield, N. J.

objective evidence (demonstrated by vaginal smears† and vaginal biopsies) of estrogen deficiency. The hormone was administered 3 to 6 times a day. Vaginal smears were taken daily and vaginal biopsies at intervals of 1 to 2 weeks.

Results. Characteristic vaginal smear changes indicating an estrogen effect were noted as early as 4 days after the beginning of hormone administration, with daily doses of 0.3 mg of α-estradiol (Figs. 1 and 2). Vaginal biopsies at the end of one week showed definite evidence of estrogen stimulation. No untoward effects were noted as a result of the propylene glycol absorption.

A number of experimental studies in animals and humans have been performed with regard to the possible toxicity of propylene

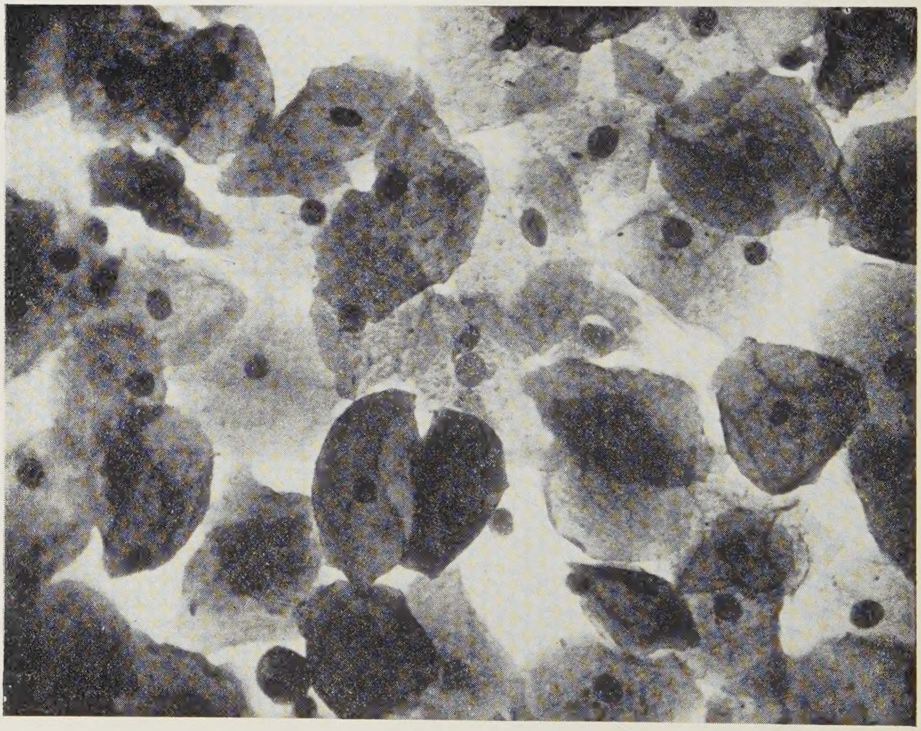


FIG. 2.

Vaginal smear taken 4 days after beginning of sub-lingual administration of α-estradiol in propylene glycol. Dose: .05 mg α-estradiol 6 times daily for 4 days = total of 1.2 mg = 14400 r.u. Smear reveals typical estrogenic effect.

---

†Vaginal smears prepared by the aqueous fuchsin technic.[7,8]

[7] Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 612.

[8] Geist, S. H., and Salmon, U. J., *Am. J. Obs. and Gyn.*, 1939, **38**, 392.

glycol.[1-6] The published reports, to date, agree as to the absence of toxic effects unless administered in large doses.

Discussion. A simple and economical method of administering estrogens for therapeutic purposes is described. This method consists of instilling several drops of a solution of α-estradiol in propylene glycol in the sublingual space. Definite morphologic evidence of absorption (demonstrated by characteristic estrogenic effects in the histologic sections of the vaginal mucosa and vaginal smears) was noted in all cases at the end of one week with daily doses of 0.2 and 0.3 mg of α-estradiol.

Although experimental studies appear to indicate that, in the small doses used, propylene glycol has no toxic effects, further studies should be conducted in order to determine what the effect of continued administration of these small doses would be. Should propylene glycol be found to be completely innocuous, this method of administration of estrogens offers great promise of simplifying and reducing the cost of estrogen therapy.

11828

Rumen Synthesis of the Vitamin B Complex.*†

M. I. Wegner, A. N. Booth, C. A. Elvehjem and E. B. Hart.
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Work reported by McElroy and Goss[1] indicates the ability of sheep to synthesize several members of the vitamin B complex in

---

[1] Weatherby, J. H., and Haag, H. B., *J. Am. Pharm. Assn.*, 1938, **27**, 466.

[2] Laug, E. P., Calvery, H. O., Morris, H. J., and Woodard, G., *J. Indust. Hyg. and Toxicol.*, 1939, **21**, 173.

[3] Hanzlik, P. J., Newman, H. W., Van Winkle, W., Lehman, A. J., and Kennedy, N. K., *J. Pharm. and Exp. Ther.*, 1939, **67**, 101.

[4] Seidenfeld, M. A., and Hanzlik, P. J., *J. Pharm. and Exp. Ther.*, 1932, **44**, 109.

[5] Hanzlik, P. J., Lehman, A. J., Van Winkle, W., and Kennedy, N. K., *J. Pharm. and Exp. Ther.*, 1939, **67**, 114.

[6] Latven, A. R., and Molitor, H., *J. Pharm. and Exp. Ther.*, 1939, **65**, 89.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

† Thanks are extended to LaVell Henderson and Dr. Harry Waisman for assistance in the pyridoxine and nicotinic acid assays, to George Bahler for biotin determinations, and to Ann Earle and Bessie Zeman for pantothenic acid and riboflavin assays.

[1] McElroy, L. W., and Goss, H., *J. Biol. Chem.*, 1939, **130**, 439.

the rumen. Significant increases in the vitamin content of rumen ingesta were found in comparison to the amounts in the ration fed. The vitamins determined were thiamin, riboflavin, pyridoxine, and pantothenic acid. The ration used consisted of washed casein 5, washed sardine meal 6.7, cerelose 11.0, corn starch 10.6, mineral mixture 3.0, and dried plain beet pulp 63.7%. In a later report[2] McElroy and Goss obtained similar results when feeding this ration to a cow with a rumen fistula.

At the time the above work was being reported a similar experiment was under way at this station. The animal used was a heifer calf with a rumen fistula. The ration fed consisted of acid-washed casein 4, urea 1.0, cod liver oil 1.0, salt mixture 3.0, corn molasses 10.0, corn starch 71.0, and bleached wood-pulp 10.0%. The calf was fed daily at 8 A.M. and 8 P.M., receiving 2 pounds of ration per feeding. After the calf had received this ration for several weeks the rumen contents were sampled on alternate days at 2 P.M. until about 20 kilos of wet material had been obtained. The size of the samples was approximately 2 kilos of wet material (12-15% dry matter). Immediately upon sampling, the material was diluted with 95% ethyl alcohol to a final concentration of 45-50% and placed in a cold room (35°F) in order to stop bacterial action. Two to 3 days later this material was placed in enamel-lined pans and dried in a drying room for 24 to 40 hours at a temperature of 45 to 50°C. After grinding these samples were again stored in the cold room and removed as needed. The analyses were assays on a composite sample.

The components of the vitamin B complex assayed for were: thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and biotin. The methods of assay used were as follows: Thiamin, chick;[3] riboflavin, microbiological;[4] nicotinic acid, chemical;[5] pyridoxine, rat;[6] pantothenic acid, microbiological;[7] biotin, microbiological.[8] The results are given in Table I.

From Table I it is readily seen that an appreciable synthesis of all members of the B complex assayed for occurs.

---

[2] McElroy, L. W., and Goss, H., *J. Biol. Chem.*, 1940, **133**, lxv.

[3] Arnold, A., and Elvehjem, C. A., *J. Nutr.*, 1938, **15**, 403.

[4] Snell, E. E., and Strong, F. M., *J. Ind. and Eng. Chem., Anal. Ed.*, 1939, **11**, 346.

[5] Melnick, D., and Field, H., Jr., *J. Biol. Chem.*, 1940, **134**, 1.

[6] Conger, T. W., *et al.*, unpublished method not yet in press.

[7] Strong, F. M., *et al.*, method unpublished. Reported to the Am. Chem. Soc., Div. Biol. Chem., 1940.

[8] Peterson, W. H., *et al.*, method unpublished. Reported to the Am. Soc. Biol. Chemists, New Orleans, March 13-16, 1940. *J. Biol. Chem.*, 1940, **133**, lxxv.

TABLE I.
Assay Results.
(γ/g dry matter.)

Factor	Basal ration	Rumen contents	Rumen contents (vitamin B_1 added to ration)
Thiamin	0	10-12	> 20
Riboflavin	< 0.4	18.6	26.5
Nicotinic acid	60	220	172
Pantothenic acid	< 3.4	55.5	82.5
Pyridoxine	0	7	11-12
Biotin	< 0.018	0.087	0.250

In a recent report by Knight, *et al.*,[9] the rapid destruction of ingested vitamin C in the rumen was demonstrated. The possibility existed that the same destruction might occur with members of the B complex. To investigate this possibility 200 mg of thiamin were added to the calf ration (given above) and the rumen contents again assayed for the members of the B complex as listed. The results are recorded in the last column of Table I. Since the samples were taken 6 hours after feeding and the thiamin content was still appreciably higher than found in the previous experiment destruction of this factor in the rumen, if any, is contraindicated. Further, the addition of thiamin to the ration apparently stimulated the synthesis of the other members of the B complex except nicotinic acid as can be readily seen by comparing the two experiments, with and without added thiamin respectively.

The results were obtained through the use of reliable methods with a possible exception of nicotinic acid, and even here the differences between the basal ration and rumen content are significant.

The synthesis of the vitamin B complex is very likely due to bacterial action in view of the stimulating effect obtained by adding thiamin to the ration fed.

Summary. The ability of the bovine species to synthesize significant amounts of thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and biotin when fed a ration very low in these compounds has been demonstrated. A destruction of thiamin in the rumen is contraindicated. At the same time there was an apparent stimulation of the synthesis of the other factors assayed for when thiamin was added to the "synthetic" ration.

---

[9] Knight, C. A., Dutcher, R. A., Guerrant, N. B., and Bechdel, S. I., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 90.

11829 P

Suitability of the Common Goldfish for Assay of Thyrotropic Hormone.

AUBREY GORBMAN. (Introduced by J. Frank Daniel.)

From the Department of Zoology and the Institute of Experimental Biology, University of California, Berkeley.

The unusual responsiveness of the thyroid tissue of goldfish to small amounts of hypophyseal thyrotropin has provided in these experiments the basis for a sensitive, inexpensive assay procedure. In a series of tests in which 115 two-inch-long fish were used, marked growth of the epithelium of the thyroid follicles was produced by crude and relatively purified preparations from the pituitaries of mammals, birds, amphibia, and fishes. One Parkes-Rowlands guinea pig unit, 0.25 mg of a purified, standardized mammalian pituitary thyrotropic powder was much larger than the minimum dose producing thyroid stimulation in the goldfish.

Groups of 5 fish receiving the same treatment were kept in one-gallon jars. The hormone was administered intraperitoneally in 5 successive daily injections of 0.2 cc each day, dissolved or suspended in the desired dilution of Ringer's solution. At autopsy, performed on the sixth day, the lower jaw was removed and trimmed. This piece of tissue, about 2 mm by 3 mm in size, was fixed in Bouin's fluid which contains enough acid to eliminate the need of special decalcifying agents The jaw was cut sagittally in 10μ sections. Only about 100 serial-sections, estimated to be approximately median sagittal and parasagittal were saved. The height of the epithelium was measured with a calibrated ocular micrometer and an average of 10 measurements from different follicles was taken.

TABLE I.
Dose of Thyrotropic Preparation Required for Specified Response, Expressed as Milligrams of Desiccated Tissue.*

	Pituitary preparations					
Reaction height epithelium, μ	Fish (sole)	Leopard frog	Bull-frog	Chicken	Sheep	Parkes Th 9
2.0μ-3.5μ	10.0	5.0	†	5.0	†	†
3.5μ-5.0μ	‡	10.0	5.0	10.0	†	†
5.0μ-9.0μ	‡	‡		10-20	150	0.25—
9.0μ+	‡	‡	20.0	‡	‡	5.0

*Excepting the Parkes thyrotropic extract for which the dose is expressed as milligrams of purified powder.

†Lowest dosage was too high to produce this response.

‡Highest dosage was too low to produce this response.

The thyroid epithelial response in this cold-blooded animal was very uniform to any given dosage level. The average control epithelial cell measured 1μ in height, with extreme variations of 0.5μ from this dimension. In strongly stimulated thyroid tissue, epithelia as high as 12μ were seen. It was found convenient to recognize different grades of stimulation. A 50% increase in average follicle cell height (cells 2μ high) was considered the minimal reaction. Increases of 175% (3.5μ), 300% (5μ), and 500% (9 to 10μ), respectively, were adjudged the arbitrary limits for delineation of the reactions referred to as mild, strong, and very strong.

11830

Further Experiments on Nutritional Achromotrichia in Rats and Mice.

Paul György and C. E. Poling.*

From the Babies and Childrens Hospital, and the Department of Pediatrics, School of Medicine, Western Reserve University, Cleveland.

From the results of previous experiments it has been concluded that "concentrates of pantothenic acid, with a purification up to 40 to 50%, appear to contain one factor but not the only factor concerned in the cure of nutritional achromotrichia in rats."[1]

Later, it became evident that this factor, which proved to be heat labile in alkaline solution, is identical with pantothenic acid.[2] In a group of rats kept on a diet free from pantothenic acid, administration of daily doses of from 75 to 100 μg of synthetic pantothenic acid† brought about cure of the nutritional achromotrichia in from 5 to 7 weeks.[2] In some rats the cure was slower and in a few it was never quite complete.

These experiments were repeated with black mice‡ kept under similar nutritional conditions. The diet used was particularly conducive[3] to the production of acrodynia in rats. It consisted of 18 parts of purified casein,§ 10 of dried heated egg white, 58 of sucrose, 8 of melted butter fat, 2 of cod liver oil and 4 of salt mixture. Raw egg white was heated over a steam bath for 3 hours in order to destroy the injurious effect ("egg white injury"[4]) it has on animals. The diet fed both to the rats and the mice was supplemented with 20 μg each of thiamin chloride, pyridoxine and riboflavin.

---

* S.M.A. Corporation Fellow in Biochemistry, assigned to the Department of Pediatrics.

[1] György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, 1940, **132**, 789.

[2] György, P., and Poling, C. E., *Science*, 1940, **92**, 202.

† Synthetic pantothenic acid was generously put at our disposal by Merck & Co. Inc., Rahway, N.J.

‡ Grateful acknowledgment is made to Dr. Agnes Fay Morgan, University of California, Berkeley, for the strain of black rats used in the experiments on nutritional achromotrichia and to Dr. William C. Barrett, Department of Anatomy, School of Medicine of Western Reserve University, Cleveland, for the C-57 strain of black mice.

In from 3 to 5 weeks, apart from the skin lesions, the fur of the mice became gray or brownish, similar in color to the fur of wild house mice or more silvery. Daily doses of from 50 to 100 μg of calcium pantothenate (synthetic, Merck) produced quick and definite effect on the depigmentation of the fur, with practical cure in from 3 to 5 weeks. The therapeutic effect on the cutaneous manifestations was even more rapid.[5] These lesions were similar to those described in rats as Type II[6] and to those recently observed in mice under similar conditions by Norris and Hauschildt.[7]

A group of 24 black rats and 8 black mice were kept under prolonged observation without the institution of any further change in the experimental conditions. They received the supplement of pantothenic acid for as long as 6 months after the initial achromotrichia had been cured.

It became evident that the diet fed these animals could not be regarded as complete. Changes in the pigment metabolism of the fur again became apparent in both the rats and the mice. In addition there were (1) cutaneous lesions in some of the rats similar to those reported by Morgan and Simms,[8] (2) a predominantly scaly dermatosis and dandruff in some of the rats and mice, and (3) a thinning of the pelt in a few rats and mice. The pathological changes in the content of pigment in the fur never reached the stage of depigmentation seen in rats and mice kept on a diet deficient in pantothenic acid, and they were characterized, as a rule, either by brownish discoloration of the black fur or by single gray hairs interspersed in the fur without generalized graying, or by both. The brownish discoloration was more conspicuous than the moderate, diffuse graying, the fur in the latter case being only slightly different from normal fur. These secondary changes appeared usually from 4 to 12 weeks after the initial nutritional achromotrichia had been cured by the administration of pantothenic acid.

---

[3] György, P., and Eckardt, R. E., *Biochem. J.*, 1940, **34**, 1143.

§ Generously furnished by the S.M.A. Corporation, Chagrin Falls, Ohio.

[4] György, P., *J. Biol. Chem.*, 1939, **131**, 733; György, P., Kuhn, R., and Lederer, E., *J. Biol. Chem.*, 1930, **131**, 745; Birch, T. W., and György, P., *J. Biol. Chem.*, 1939, **131**, 761.

[5] György, P., and Poling, C. E., data to be published.

[6] György, P., and Eckardt, R. E., *Nature* (London), 1939, **144**, 512.

[7] Norris, E. R., and Hauschildt, J., *Science*, 1940, **92**, 316.

[8] Morgan, A. F., and Simms, H. D., *J. Nutrition*, 1940, **19**, 233.

When 100 μg of a highly purified concentrate of biotin[9] were added to the daily diet fed this group of rats and mice, or 0.25 μg of crystalline biotin methyl ester[‖] to the diet of 2 rats, definite and quick improvement in the pigment metabolism of the fur was noted.

These observations are in agreement with those made in rats kept on a diet that produced typical egg white injury,[10] the pelts of which showed distinct signs of depigmentation, such as mild graying, brownish discoloration and rusting. The present group of rats and mice was fed a diet which contained only a small amount of egg white (10%) and even this amount was detoxified to a large extent by heating. However, the possibility that it may have affected the pigment metabolism of the pelt cannot be denied. Be that as it may, the experiments here reported prove that there is a possible interrelationship between biotin and pigment metabolism of the fur in rats and mice.

The order in which pantothenic acid and biotin were administered was found to be of decisive importance in the present series of experiments. When supplements of biotin were given before supplements of pantothenic acid, achromotrichia was somewhat aggravated, and the curative effect of biotin became evident only when it was given during the period of secondary changes in the fur, after the initial achromotrichia had been relieved by pantothenic acid.

In black rats and mice treated with pantothenic acid and biotin, pigmentation of the fur still did not necessarily become entirely normal. It should be borne in mind, however, that the fur of black rats kept on the stock diet[11] shows, as a rule, with progressing age, a slight brownish discoloration and is often interspersed with single gray hairs. The question arises whether this condition should be considered a function of age rather than one of incomplete diet.

Conclusion. Pantothenic acid is only one although probably the most important factor in the prevention of nutritional achromotrichia as it is seen in rats and mice used in experiments on the vitamin B_2 complex. Biotin is an additional factor that is connected with the maintenance of the normal pigment metabolism of the fur in rats and mice under special dietary conditions.

---

[9] du Vigneaud, V., Melville, D. B., György, P., and Rose, C. S., *Science*, 1940, **92**, 62.

[‖] Kindly furnished by Professor F. Kögl, Utrecht, Netherlands.

[10] György, P., in Pfaundler, A., and Schlossmann, M. v., *Handbuch der Kinderheilkunde*, 1935, **10**, 55.

[11] Sherman, H. C., and Muhlfeld, M., *J. Biol. Chem.*, 1922, **53**, 41; Smith, A. H., and Bing, F. C., *J. Nutrition*, 1928, **1**, 179.

11831

Effects of Epinephrine and Amphetamine on Respiration and Blood Pressure in Different Postures.

ROLLAND J. MAIN.

From the Department of Physiology, Medical College of Virginia, Richmond.

The cause of the overventilation of the erect posture, as indicated by lowering of the alveolar CO_2 tension, is unknown. Main[1] suggested that it might be due to the fact that the pressure in the carotid sinus is lowered about 20 mm Hg on standing,[2] which should therefore stimulate respiration. Turner[3] believed it might be due to cerebral ischemia. I decided to test the former hypothesis by raising the blood pressure of a standing subject, to see if this would remove the excess respiratory stimulation by increasing the pressure in the carotid sinus back to the normal level.

I decided to use epinephrine subcutaneously to raise the blood pressure. Two reports in the literature on the effect of epinephrine on alveolar CO_2 are somewhat at variance; Arnoldi[4] reporting a rise, and Peters,[5] a slight fall. However, the side effects of this drug, such as marked tremor, cold perspiration, feeling of apprehension, and the possible production of a lactacidemia,[6] made it advisable to control the results with some other vasopressor drug which would not produce such deleterious side effects. Amphetamine sulfate subcutaneously was selected because of its entirely different side effects: euphoria, and the complete lack of tremor and sweating.

It was soon found that the subjects, males between the ages of 20 and 30, varied greatly in their response to epinephrine; some being severely affected by 0.5 cc, and others showing very little ill effects from 0.75 cc of a 1 to 1000 solution in ampules (Parke-Davis). No ill effects were noticed in any case from amphetamine sulfate in doses up to 30 mg. Care was always taken not to make an accidental intravenous injection, and not to massage the area after injection. Not more than one injection a week was given to a subject, in order to prevent any possible adaptation. A total of 12 subjects was used, in 12 experiments with amphetamine, and 24 with epinephrine.

---

[1] Main, R., *Va. Med. Monthly*, 1937, **64**, 330.

[2] Loman, J., and Myerson, A., *Am. J. Psychiat.*, 1936, **92**, 791.

[3] Turner, A., *Am. J. Physiol.*, 1927, **80**, 601.

[4] Arnoldi, W., *Deut. Med. Woch.*, 1924, **50**, 1397.

[5] Peters, J., *Am. J. Physiol.*, 1917, **44**, 84.

[6] Cori, C., *J. Biol. Chem.*, 1925, **63**, 253.

After eliminating a few subjects who were very sensitive to the effects of epinephrine, I found it necessary to administer from 0.5 to 0.75 cc of a 1 to 1000 solution, in order to obtain a maximal increase in systolic blood pressure of about 20 mm Hg. From 20 to 30 mg of amphetamine sulfate subcutaneously was required to produce a similar effect.

The subjects came to the laboratory within an hour after having eaten, so that hunger contractions would not intervene toward the end of the experiment to disturb the CO_2 tension.[7] They were not permitted to have coffee at the previous meal or to smoke one hour beforehand. They would lie down for at least 30 minutes before the experiment was started. The effect of the drug was tested in the supine position the entire period, or at other times was given while standing.

Alveolar CO_2 samples were usually taken every 10 minutes to cover normal variations in tension, and unconscious errors in obtaining the sample. The Haldane-Priestley method was used; the forced expiration being made at the end of a normal expiration.

Fig. 1 illustrates the typical effect of epinephrine (0.6 cc subcutaneously) on the same subject, lying and standing. With the doses of epinephrine used throughout (0.25 to 0.75 cc), there was never noted any distinct effect upon alveolar CO_2 tension, even when the blood pressure of the standing subject rose over 20 mm Hg. Apparently any possible lactacidemia produced by these doses of epinephrine is inadequate to affect the acid-base balance of the blood. Neither the distinct tremor nor the apprehension produced by the drug seemed to have any effect upon the CO_2 tension.

The effect of amphetamine sulfate was tested in the same way as with epinephrine. There was always a distinct feeling of euphoria which lasted less than 2 hours and had disappeared by the end of the experiment. No effect on alveolar CO_2 was noticed with this drug, even when it caused a distinct increase in blood pressure.

I would estimate, that from its maximum effect on blood pressure (but not duration), that 20 to 25 mg of amphetamine sulfate is equivalent to about 0.6 cc of 1/1000 epinephrine, by subcutaneous administration.

---

[7] Main, R., *Am. J. Physiol.*, 1937, **119**, 7.

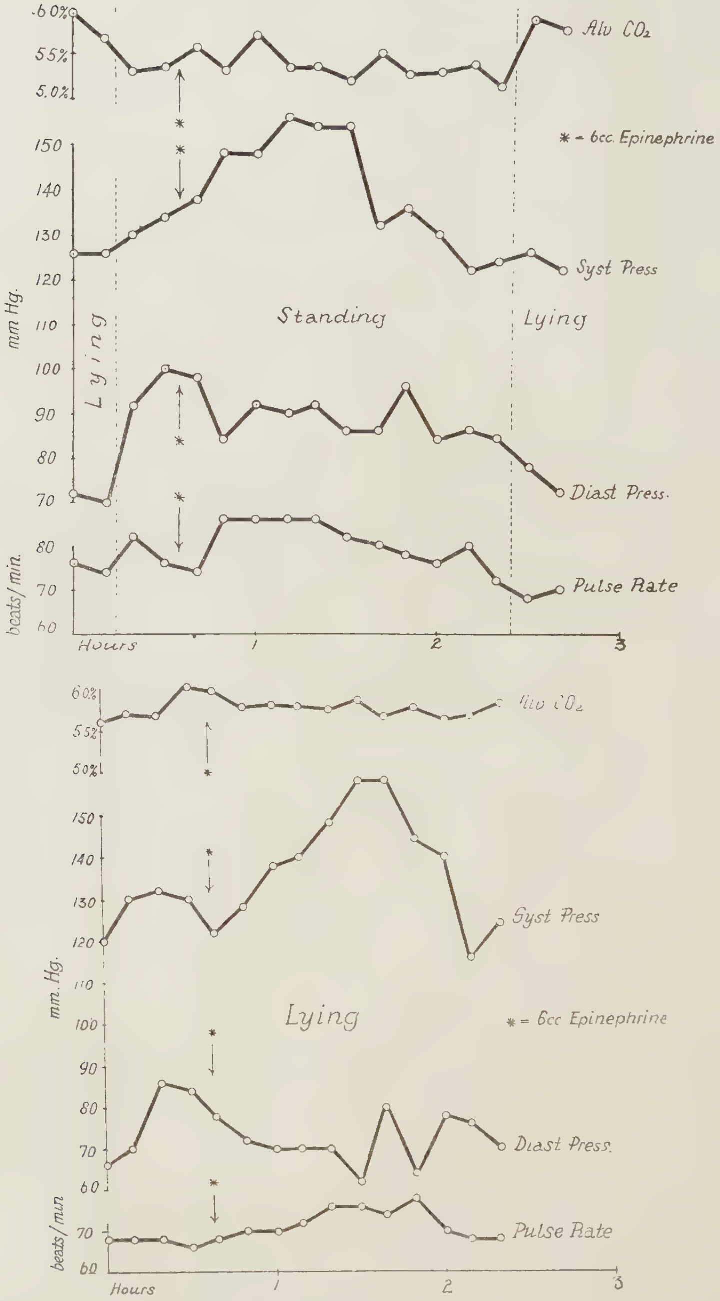


FIG. 1.

Effects of 0.6 cc epinephrine (1-1000) subcutaneously on the same subject in different postures.

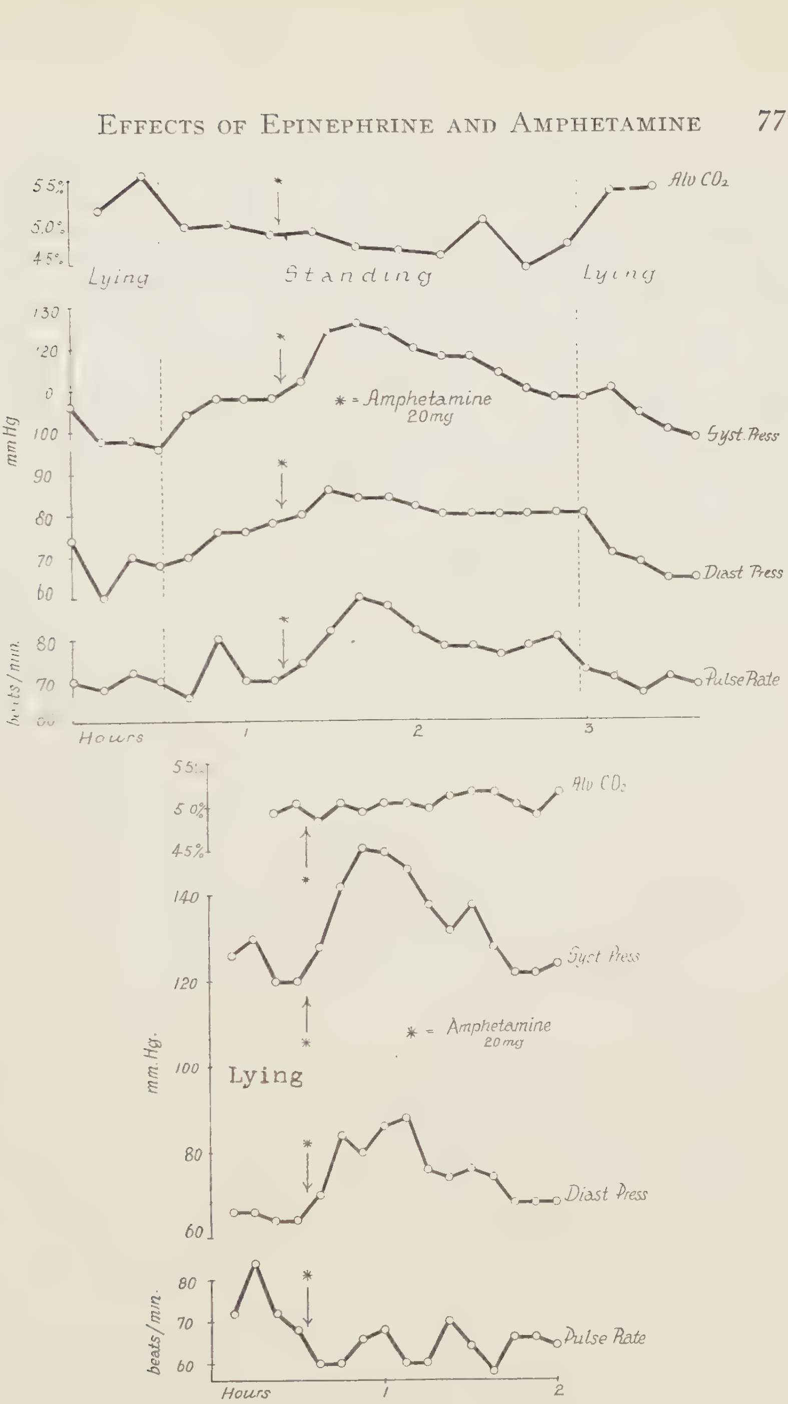


FIG. 2.

Effects of 20 mg amphetamine sulfate subcutaneously to one subject in different postures.

Fig. 2 illustrates the effect of 20 mg of amphetamine sulfate on the same subject, lying and standing. Considerable variation was found, even in the same individual from day to day. A dose that would produce a rise of 20 mm Hg in blood pressure in one day, might produce a rise of only half as much the next week. Neither drug had a distinct effect upon the respiratory rate.

Since increasing the systemic blood pressure and thereby probably restoring the normal pressure in the carotid sinus, did not restore the alveolar CO_2 to normal, we must assume that the overventilation of standing may not be due to the drop in blood pressure in the carotid sinus and we are forced back to the remaining hypothesis, that the respiratory stimulation is due to a relative ischemia of the brain. These results would indicate that the rise in alveolar CO_2 on bending over while standing,[7] may be due not to the return to normal pressure in the carotid sinus, but rather to relief of the cerebral ischemia. It at first seemed illogical that the drop in blood pressure in the carotid sinus on standing should not cause respiratory stimulation, but it may be that the probable *increase* of pressure in the aorta on standing exactly neutralizes the *drop* in blood pressure in the carotid sinus.

Conclusion. Neither epinephrine nor amphetamine in the doses used affected the alveolar CO_2 tension of subjects when standing or lying. Consequently the lowered alveolar CO_2 of the erect posture may be due to a relative cerebral ischemia.

This work was made possible by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

11832

Twenty-four Hour Response to Androgens in the Immature Male Rat.*

R. R. GREENE AND M. W. BURRILL. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Astwood[1] has demonstrated that estrogens cause a measurable increase in the uterine weight of the immature rat within 6 hours after administration. The weight increment which is largely due to increased hydration of the uterine tissues forms the basis for his estrogen assay method. Astwood later stated[2] that androgens have the same effect on the rat uterus. This latter finding has been confirmed.[3] The present investigation was undertaken to determine whether or not androgens have a similar weight-increasing effect on the sexual accessories of the immature male rat.

---

* Aided by a grant from Ciba Pharmaceutical Products, Inc.

[1] Astwood, E. B., *Endocrinology*, 1938, **23**, 25.

One hundred and thirty-one male rats, 32 to 38 days old and 65 to 85 g in weight, were used. Twenty-one animals served as controls. The rest were divided into 5 groups which were given various doses of testosterone propionate† in 0.1 cc of peanut oil in a single injection subcutaneously. Twenty-four hours after treatment the animals were killed. The ventral prostates and seminal vesicles were carefully removed under a dissecting microscope and immediately weighed on a Roller-Smith micro-balance. The dosages used and the average weights of the removed organs are presented in tabular form (Table I).

With the exception of the lowest dosage group (0.00625 mg) the prostates showed a uniform and progressive increase in mean weights with increasing dose. The seminal vesicles showed a relatively greater response to androgens in the 24-hour period. The increase in mean seminal vesicle weight was consistent with the increased dose given.

The mean weights of both prostates and seminal vesicles obtained with 0.5 mg testosterone propionate were significantly greater than those of the controls (critical ratios of 3.36 for the prostates and 4.63 for the seminal vesicles). The mean seminal vesicle weight with 0.1 mg testosterone propionate was also significantly greater than that of the control (critical ratio 3.64) but the mean prostate weight at this dose was not. The differences between the lower dosage groups and the control group were not significant.

TABLE I.

Test. prop. mg	No. animals	M. body wt	M. prost. wt		% increase over cont.	M. sem. ves. wt		% increase over cont.
			mg	σ		mg	σ	
—	21	75.0	45.90	2.38	—	12.08	0.87	—
0.00625	25	74.0	42.62	1.80	—7.14	13.36	0.62	10.59
0.025	20	73.9	47.85	2.22	4.24	14.43	1.06	19.46
0.5	25	73.8	48.23	2.12	5.07	15.15	0.87	25.41
0.10	20	73.5	51.53	2.01	12.26	*16.49	0.83	36.51
0.05	20	74.5	59.95	2.38	*30.61	*18.54	1.08	53.47

σ = Standard deviation of the mean.
* = Significant difference from control mean.

---

[2] Astwood, E. B., *Proc. Am. Physiol. Soc.*, 1940, p. 6.

[3] Greene, R. R., and Harris, S. C., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 34.

† Testosterone propionate (Perandren) furnished by courtesy of Ciba Pharmaceutical Products, Inc.

Within groups the individual prostate and seminal vesicle weights varied considerably. Since the animals used in this procedure also varied in age (32 to 38 days) and in weight (65 to 85 g) it seems probable that some of the intragroup variation in weight of the accessories may be due to these factors. In spite of the intragroup variation, however, the mean seminal vesicle weights in the treated groups showed a fairly consistent direct relationship to the logarithm of the dose as is characteristic of biological responses.

It is possible that a procedure similar to this might be useful as an assay method. The matter of greater accuracy (less group variability), the specificity of the response and the possibility of a shorter time period are being investigated.

Summary. Twenty-four hours after the administration of testosterone propionate to the immature male rat there is an increase in the weights of the prostate and seminal vesicle. This weight increment is proportional to the dosage used.

11833 P

Infusion of Blood and Other Fluids into the Circulation via the Bone Marrow.

L. M. Tocantins and J. F. O'Neill.

From Division of Hematology and Surgical Service B, the Jefferson Medical College and Hospital, Philadelphia.

Substances injected into the bone marrow enter the general circulation apparently unchanged and almost as rapidly as when injected intravenously.[1] In the parenteral administration of fluids to adult men it is sometimes impossible to use the intravenous route. Such is the case in widespread mutilations, burns, oedema, poorly developed, obliterated veins, states of shock. In the newborn and in early infancy the venous system is so poorly developed that the superior longitudinal sinus reached through the anterior fontanelle is often the only available direct path to the circulation. The risk of injecting substances through this route is high. These facts seemed to justify an attempt at a wide clinical application in man of the intramedullary route for parenteral therapy. Trial of this route has been carried out in 14 persons. Ten infusions of citrated blood were given by this method to 7 patients, plasma infusions to 2 patients and infusions of 5% glucose and salt solution to 4 patients. In 17 trials there was one failure (Case 10); little marrow could be aspirated from the sternum and no fluid could be injected by gravity. In 2 children the fluids were introduced into the tibia and femur. In all the other patients the sternum or the clavicle were used. From 100 to 1050 cc have been injected, the infusion needle being left in place for a duration of from 1½ to 16 hours. Some signs of discomfort are evident during the first steps of the procedure (aspiration of marrow and injection of salt solution with a syringe). Throughout the course of the infusion practically no discomfort has been felt. The average rate of infusion varied between 0.4 and 9 cc per minute. Strict asepsis has been observed throughout. Indications that the infused citrated blood had been rapidly absorbed were a substantial increase in the amount of hemoglobin and number of erythrocytes in the blood of the patients 24 hours after the infusion. Readings of the intramedullary pressure as obtained with a water manometer before the start and after completion of the infusion have not differed by a significant margin. The readings varied between 50 and 120 mm of water and often corresponded closely with readings of the pressure in the veins of the forearm. There have been no local or constitutional reactions as evidenced by clinical or X-ray findings following any of the infusions. The 2 children that received the blood infusions were under one year of age and in neither of them were there veins available for transfusions. The method and apparatus used and the results obtained will be described in detail elsewhere.

---

[1] Tocantins, L. M., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 292.

Within the limitations outlined the intramedullary route for the parenteral administration of blood and other fluids seems to have its indications and has proved to be feasible. Application of the method should be limited to such times as when the intravenous route is not available and only by those familiar with the technic and favorable points of approach. Work now in progress indicates that other bones may have equal or greater advantages over those previously employed.

Summary. The intramedullary route for parenteral therapy has proved practicable in 16 out of 17 trials in 14 patients. Citrated blood, plasma, glucose and salt solutions have been infused without any immediate or delayed local or constitutional reactions.

11834 P

Tissue Ascorbic Acid in Hypophysectomized Rats.

DONALD E. BOWMAN, LUCIEN E. MORRIS AND JOHN R. STACY.
(Introduced by Edward Muntwyler.)

From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland, Ohio.

Some time ago it was observed that the post-operative administration of crystalline ascorbic acid to hypophysectomized rats caused a very marked and sudden improvement in some cases in which recovery otherwise appeared quite doubtful. The tissue ascorbic acid was estimated in some of the untreated rats which failed to recover and it was found to be very low. Although the full significance of these preliminary observations is not clear a brief account may be of interest.

The 6-week-old rats were maintained since weaning entirely on Ralston Purina Dog Chow which was found to be scorbutigenic for guinea pigs. The ascorbic acid of the tissues was estimated in the usual manner by titrating a metaphosphoric acid extract of the tissues with 2,6-dichlorophenol indophenol. That the tissue ascorbic acid values in the 5 hypophysectomized rats examined are definitely below normal is indicated by the data of Table I. Some of the rats had received daily glucose injections and in all cases the stomachs contained food at autopsy. Hemorrhagic areas in various degrees of severity were also observed. The corresponding values for control, non-hypophysectomized, normal rats, determined at the same time, fell within the normal limits.

These findings indicate that a dietary intake or the injection of ascorbic acid after hypophysectomy in rats may prove highly beneficial. A more detailed study of the ascorbic acid metabolism of such animals is proposed.

TABLE I.
Tissue Ascorbic Acid Values in Hypophysectomized and Normal Rats.

	mg per 100 g of tissue			
	Liver	Kidney	Adrenal	Small intestine
Avg 5 hypophysectomized rats	8.36	6.5	160.8	4.8
Max.	10.25	8.2	164	9.6
Min.	7.2	4.8	89.8	2.0
Avg for 5 normal rats	23.2	15.2	340	21.2
Max.	30.1	19.1	450	26.4
Min.	16	12.3	150	16.2

11835

Chromodacryorrhea and Parasympathetic Action of Cyclopropane.

JOHN ADRIANI, STEVENS J. MARTIN AND E. A. ROVENSTINE.
From the Division of Surgery, Department of Anesthesia, New York University College of Medicine, New York City.

The effects of anesthetic drugs upon the autonomic nervous system are of recent interest. Those of ether upon the sympathetic division have been described.[1] Parasympathetic stimulation by cyclopropane has been suggested from laboratory observations in dogs.[2] The results of a study of this latter action and its *modus operandi* are contained in this report. Cyclopropane does not inhibit the activity of cholinesterase of human serum *in vitro*.[3] Two of us[4] have noted that perfusion of turtles' and frogs' hearts with solutions of cyclopropane produced parasympathetic effects. Perfusates from eserinized stomachs and hearts exhausted of the cyclopropane likewise produced parasympathetic stimulation when added to fresh eserinized hearts or to preparations of the frog's rectus abdominis muscle. Such results suggested the liberation of an acetylcholine-like substance. These results were not obtained with ethylene and ether. Recently Tashiro, *et al.*,[5] described a technic for the assay of acetylcholine by the response of chromodacryorrhea or "red tears" in the eserinized albino rat. This procedure was utilized here to demonstrate further the parasympathetic action of cyclopropane.

Procedure and Results. Sixty-one observations were made on 36 white adult male rats weighing 225 to 325 g. Fifty gamma of eserine salicylate per 100 g of rat were given intraperitoneally. Twenty minutes later ether, cyclopropane, or ethylene narcosis was induced and maintained in the first plane of surgical anesthesia for a minimum of 20 minutes. The eyes were examined for the presence of chromodacryorrhea: (1) 20 minutes after the injection of eserine before anesthesia was begun, (2) immediately after anesthesia was discontinued, and (3) 20 minutes after recovery. Thirty control observations were made on non-premedicated, unanesthetized rats

---

[1] Bhatia, B. B., and Burn, J. H., *J. Physiol.*, 1933, **78**, 257.

[2] Seevers, M. H., Meek, W. J., Rovenstine, E. A., and Stiles, J. A., *J. Pharm. and Exp. Therap.*, 1934, **51**, 1.

[3] Adriani, J., and Rovenstine, E. A., *Anes. and Anal.*, 1940, in press.

[4] Adriani, J., and Rovenstine, E. A., *Am. J. Physiol.*, 1940, **129**, 299.

[5] Tashiro, S., Smith, C., Badger, E., and Kezur, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44** 658.

TABLE I.
Chromodacryorrhea in Eserinized Rats.

Anesthetic agent	Premed- ication	Observations No.	No. of rats showing chromodacryorrhea		
			Before Anesthesia	At end of Anesthesia	Twenty minutes after recovery
None	Eserine	64	3	0	0
Cyclopropane	None	18	0	0	0
Ether	,,	12	0	0	0
Cyclopropane	Eserine	23	0	17	1
Ethylene	,,	18	0	0	1
Ether	,,	20	0	0	2

and on those anesthetized with cyclopropane and ether without the administration of eserine. Twelve rats were treated with acetylcholine and the occurrence of chromodacryorrhea noted, thus confirming the studies of Tashiro, *et al.*[5]

A positive response for chromodacryorrhea was obtained in 18 of 23 eserinized rats treated with cyclopropane (Table I). This response was noted immediately after anesthesia was terminated. It was delayed in one rat which showed a positive response 20 minutes after recovery. The response was negative in the remaining 5 anesthetized with cyclopropane. Two of the 20 rats anesthetized with ether and one of the 18 treated with ethylene developed a positive response. Three unanesthetized eserinized rats similarly showed chromodacryorrhea. In none of the non-premedicated unanesthetized rats or the non-premedicated rats anesthetized with cyclopropane or ether was chromodacryorrhea observed.

Discussion. Since chromodacryorrhea follows the injection of acetylcholine in eserinized rats,[5] the positive responses obtained from cyclopropane suggest that this drug liberates acetylcholine in rat tissue. The exceptional results obtained with the eserinized rats anesthetized with ether and ethylene may be due possibly to an increased acetylcholine secretion incident to handling or to variations in individual threshold since chromodacryorrhea may be elicited with larger doses of eserine. This may also account for the absences of chromodacryorrhea in the 5 eserinized rats given cyclopropane.

Summary. The phenomenon of chromodacryorrhea produced by cyclopropane in eserinized white rats indicates that acetylcholine is liberated in mammalian tissues and supports the contention that the drug is a parasympathetic stimulant.

11836

Physical Allergy and "Auto-Antigens."

Edward H. Richardson, Jr. (Introduced by Arnold R. Rich.)

From the Department of Pathology, The Johns Hopkins Medical School, Baltimore, Md.

In an attempt to determine whether physical allergies could be explained on an immunological basis Karady,[1] in recent experiments, subjected a number of guinea pigs to heat and another group to cold in the manner described below. He found that a re-exposure to the same stimulus at the end of 2 weeks produced anaphylactic shock in the animals so treated, and in some of the guinea pigs the shock was severe enough to cause death. He also injected guinea pigs with guinea pig serum that had been subjected to heat and others with serum that had been subjected to cold. After 2 weeks the reinjection of heated or chilled serum into the animals that had previously been injected with heated or chilled serum respectively produced signs of anaphylaxis, and at autopsy the animals showed the pulmonary emphysema characteristic of anaphylactic shock. On the basis of these results Karady suggested that a physical stimulus, such as heat or cold, might so alter the protein of the blood plasma as to form an "auto-antigen" within the animal's own body, so that a re-exposure to the same stimulus would cause anaphylactic shock as in the above experiments. It was thought desirable to repeat these experiments and, if they could be confirmed, to study the mechanism of the sensitization. To this end we used the methods described by Karady, as follows:

Serum was obtained from normal guinea pigs, and half was heated at 56°C for 1.5 minutes and the other half cooled to –5°C for 1.5 minutes. Three cc of the heated serum was then injected into each of 7 guinea pigs by the intraäbdominal route and 7 guinea pigs were injected with the same amount of cold-treated serum. When these injections were repeated at the end of 2 weeks the animals were observed closely for an hour following the injection and during that period no signs of anaphylaxis were seen in any of them. On the contrary their behavior seemed perfectly normal, and all survived.

In our next experiment, following the procedure of Karady, we subjected 12 male guinea pigs (which were somewhat larger than those used by Karady) to cold by immersing their hind limbs in an ice-salt mixture at –5°C for 2 minutes. At the end of 2 weeks the immersion was repeated without any apparent effect on the animals which were carefully observed for an hour following the second exposure. All of them survived.

---

[1] Karady, S., *J. Immunol.*, 1939, **37**, 457.

In order to be certain that the size of the animals had nothing to do with their lack of response to this treatment we repeated this portion of the experiment using 10 male guinea pigs all of which weighed between 200 and 250 g (the size of those used by Karady). Again the second exposure was entirely without effect in producing any signs of shock. After another lapse of 2 weeks their hind limbs were again immersed in the ice-salt mixture. Following this third exposure they were carefully observed for a period of one hour and once more failed to show any anaphylactic response. All animals survived.

Finally, another group of 7 male guinea pigs (weighing 200 to 250 g) were subjected to heat by immersing their hind limbs in water at 56°C for 2 minutes. Three weeks later they were again subjected to the same treatment, but no signs of anaphylaxis were observed in any of them during the hour following the re-exposure, and all survived.

Summary. We have been unable to confirm the report of Karady that guinea pigs can be sensitized anaphylactically to heat or cold by heating or chilling their limbs. We were also unable to confirm Karady's report that guinea pigs can be sensitized to heated or chilled guinea pig serum.

11837

Uterine and Extrauterine Localizations of Experimental Fibroids Induced in Guinea Pig by Prolonged Administration of Estrogens.*

ALEXANDER LIPSCHÜTZ, RIGOBERTO IGLESIAS AND LUIS VARGAS JUN. (Introduced by Emil Witschi.)

From the Department of Experimental Medicine, National Health Service of the Republic of Chile, Santiago.

The occurrence of uterine fibroids was reported in a number of guinea pigs treated for several months with estrogens.[1] *Extrauterine*

---

* This investigation has been aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, The Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation, and Mr. Adolfo Eastman of Limache (Chile). The esterified estrogens were generously supplied by Dr. Carl Miescher of Messers Ciba, Basel; and the artificial ones by Professor Dodds, London.

abdominal fibroids also were induced in guinea pigs injected subcutaneously with estrogens in the course of 4 months.[2] Fibroids appear even if quantities as small as 5γ of the monobenzoic ester of estradiol per injection or a total of 400γ are given in the course of 3 months.† All estrogens, free or esterified, natural or synthetic, proved to be tumorigenic.[3] Tablets of estradiol introduced beneath the skin may induce fibroids as early as in 3 weeks.[4] There is an enormous variability as to the localization of uterine and extrauterine fibroids, although when a sufficient number of animals is observed, some typical localizations can be established. A survey of these localizations of experimental fibroids is given in the present paper (Figs. 1-16) based on the results of the autopsy of several hundred females to which the following 10 estrogens were administered: estrone, estriol, estradiol, 4 esters of the latter (monobenzoate, monocaprylate, dipropionate, 17-benzoate-3-n-butyrate), stilbestrol, dipropionate of stilbestrol, hexestrol.

Uterine tumors are mostly subserous (Figs. 3, 4, 14-C) and can become pedunculated (Fig. 2). Very often they are to be found in the mesometrium (Figs. 6, 8); intramural fibroids and those of the submucosa are rare (Fig. 9). The whole surface of the uterine stem and part of the uterine horns may be covered by an extensive fibroid (Fig. 1). The angle between the horns is often the site of a tumor. There may be also a chain of small tumors on the ventral surface of the uterus (Fig. 5).

Subserous fibroids may be present also on the vagina and very rarely in the vaginal submucosa (Fig. 10). In the *castrate* guinea pig an almost constant site of fibroids is near the upper ends of the tubes (Figs. 4, 5); these "apical" tumors probably originate in the mesosalpinx and can attain an enormous size (Figs. 7, 14-C), especially when joining with neighboring tumors of the spleen (Fig. 4) or the abdominal wall. The apical tumor can descend also into the pelvis (Fig. 14-C). Sometimes enormous tumoral masses en-

---

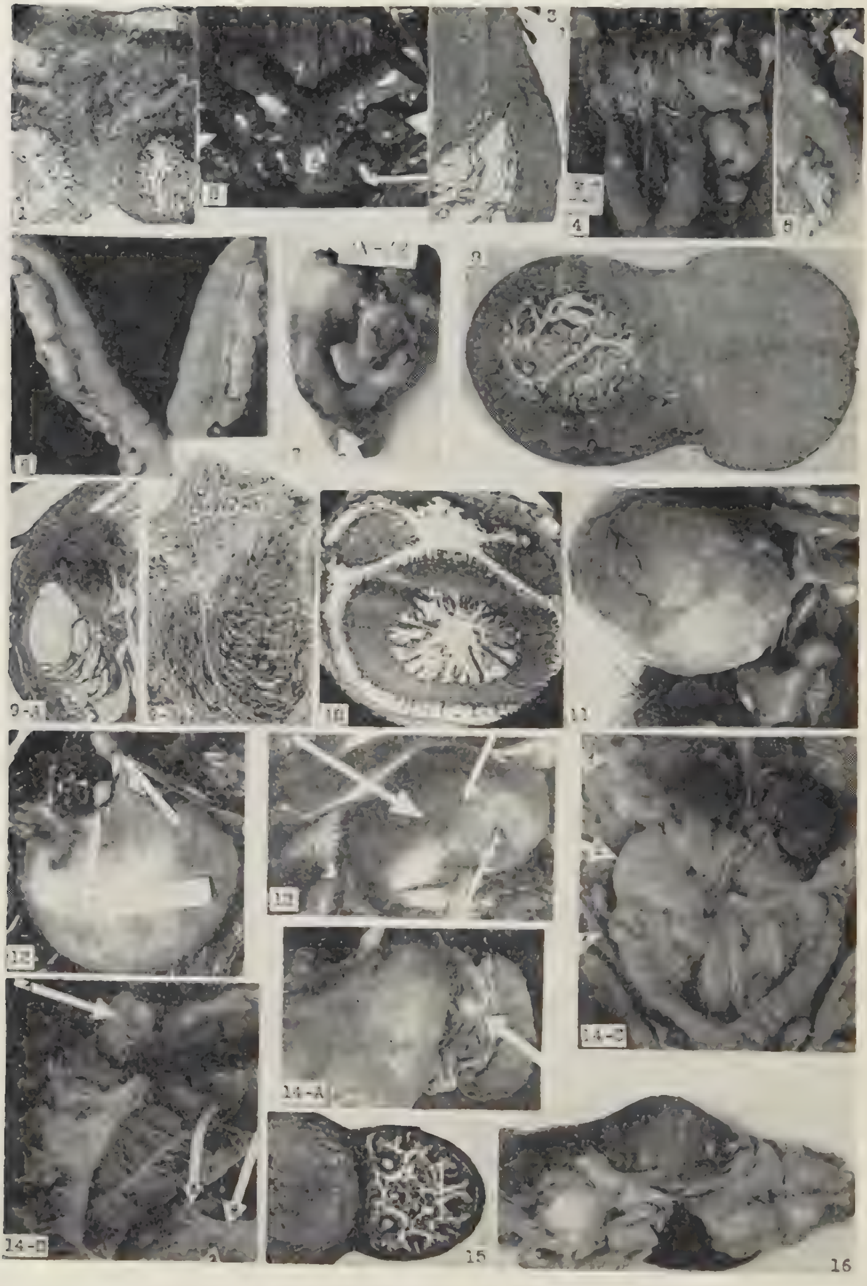
[1] Nelson, W. O., *Anat. Rec.* 1937, **68**, 99; *Endocrinology*, 1939, **24**, 50; Moricard, R., and Cauchoix, J., *C. R. Soc. Biol.* (Paris), 1938, **129**, 556.

[2] Lipschütz, A., and Iglesias, R., *C. R. Soc. Biol.* (Paris), 1938, **129**, 519; Iglesias, R., *Public. Med. Exp.* (Chile), 1938.

† Lately we have found that fibroids can be induced by as little as 2γ per injection or a total of only 80γ of estradiol in the course of 3 months when the monocaprylic ester is used (Lipschütz, Vargas, Baeza-Rosales and Baeza Herrera, to be published soon).

[3] See Lipschütz, Vargas, Koref, Jedlicky, Bellolio, Murillo, Rodríguez, Chaume, Szabó, Ruz, *C. R. Soc. Biol.* (Paris), 1938-1939, **129**, 131; *The Lancet*, 1939-1940; *Public. Med. Exp.* (Chile), 1939-1940; *Rev. Chil. Hig. y Med. Prevent.*, in press.

[4] Lipschütz, A., and Vargas, L., Jr., *The Lancet*, 1939, **1**, 1313.



EXPLANATION OF FIGURES.

In each figure explanation three data are given: the first indicates the quantity per injection, the second the total amount and the third the duration of treatments.

FIG. 1. Estradiol-monobenzoate 40γ (total 2.1 mg) 125 days. Tumor on the

dorsal face of the uterus. No sharp limit between tumor and myometrium. Adenomatous condition of endometrium. × 5.

FIG. 2. Estradiol-monobenzoate 20γ (total 5.8 mg) 183 days. Three pedunculated tumors of uterus.

FIG. 3. Estradiol-monobenzoate 20γ (total 2.6 mg) 310 days. Large subserous tumor with deformation of myometrium. × 5.

FIG. 4. Estradiol-monocaprylate 10γ (total 0.4 mg) 90 days. Subserous uterine tumor. Apical tumors on both horns; the left one with ramifications covering left kidney and adhering to spleen, pancreas and abdominal wall.

FIG. 5. Estradiol-monocaprylate 10γ (total 0.4 mg) 108 days. Chain of subserous tumors on the right horn; the chain corresponds to the muscular elevation present in the normal uterus. Arrow points to small apical tumor.

FIG. 6. Estradiol-monocaprylate 10γ (total 0.4 mg) 91 days. Mesometrial tumors.

FIG. 7. Estradiol-monobenzoate 80γ (total 2.4 mg) 85 days. Apical tumors on both sides. Partial necrosis of uterine horns (arrow).

FIG. 8. Estradiol 400γ (total 20.8 mg) 123 days. No sharp limit between myometrium and mesometrial tumor. Polyps in the uterine cavity. × 5.

FIG. 9. Estradiol 17-benzoate-3-n-butyrate 10γ (total 0.4 mg) 91 days. A—''Fibroids'' of the uterine submucosa. Cystic glandular hyperplasia. × 5. B—Same preparation. × 45. No sharp limit between ''tumor'' and stroma of uterine mucosa.

FIG. 10. Estradiol-monobenzoate 20γ (total 2.6 mg) 310 days. Uterine cervix surrounded by vagina. Two vaginal fibroids, one pedunculated. × 5.

FIG. 11. Estradiol-monobenzoate 80γ (total 3.2 mg) 110 days. Cystic dilatation of right tube with thick fibrous masses in the wall of the cyst. Apical tumor on the left (upper half of left uterus cut away).

FIG. 12. Estradiol-monobenzoate 40γ (total 2.1 mg) 124 days. Tumor near cardias, and tumor of small curvature.

FIG. 13. Estradiol-monobenzoate 80γ (total 5.8 mg) 183 days. Subserous tumors of the stomach.

FIG. 14. Estradiol-monobenzoate 80γ (3.0 mg) 88 days. A—Tumor in the hilum of spleen (arrow). B—Mesenteric tumor (arrow 1). Fibrous strands in the mesentery (between arrows 2, 3 and 4, with small tumor of the intestine (arrow 3). C—Apical tumors, the right one enormous (arrow 2). Subserous uterine tumor (arrow 3). Tumor between diaphragm and abdominal wall (arrow 1).

FIG. 15. Estradiol-monobenzoate 80γ (total 3.9 mg) 122 days. Mesenteric tumor. × 5.

FIG. 16. Estradiol-monocaprylate 10γ (total 0.4 mg) 90 days. Tumors in the hilum of the spleen.

globe the upper third or more of the uterine horn (Fig. 7). There is often no sharp limit between the myometrium and the tumor (Figs. 1, 8). Sometimes the myometrium is deformed by the subserous tumor (Fig. 3). The fibroids of the submucosa are still less delimited (Fig. 9-B).

The subserous *extrauterine* tumors are to be found on most of the abdominal organs: on the stomach (Figs. 12, 13), spleen (Figs. 14-A, 16), pancreas, liver, kidney, urinary bladder; in the epiploon, in the mesentery from the pylorus to the rectum (Figs. 14-B, 15); on the abdominal wall (Figs. 4, 13) and the diaphragm (Fig. 14-C). Very frequently fibrous strands visible to the naked eye can be found on the abdominal wall, the mesentery and other parts of the abdominal cavity.

The uterine or extrauterine localizations have no connection with lymphatic glands.

There is no case with tumors in the thoracic cavity. Tumors present on the abdominal side of the diaphragm may infiltrate between muscle fibres, but the thoracic side of the diaphragm remains always smooth and free of fibrous nodules. No tumors are found at the site of injection and only very exceptionally at the site of subcutaneous implantation of a tablet of estradiol.

11838 P

Studies on Fluorescence Associated with Proteins.

WENDELL REEDER AND V. E. NELSON.
From the Laboratories of Physiological Chemistry, Iowa State College.

The relative fluorescence intensities of the proteins and their hydrolysates were determined by measuring the dilution required to reduce the fluorescence of a given amount of protein or protein hydrolysate to the same intensity as the fluorescence of a diluted standard solution of quinine bisulfate.

The proteins prepared and studied in this investigation were: casein, wheat gluten, gliadin, glutenin, blood fibrin, gelatin, ovalbumin and zein. Hair and wool were also compared to the above proteins.

When examined in ultraviolet light of wavelengths 3100-4100 Å the proteins give a uniform bluish-white fluorescence in the solid state and a somewhat more green fluorescence in solutions. The fluorescence of these proteins is more green in basic solution than in acid, but the color change is not sharp.

Fluorescence of proteins is destroyed by oxidation with strong nitric acid or by ashing. The small amount of protein ash is not fluorescent in the solid state nor in acid, basic or neutral solution.

Organic solvents do not extract the fluorescent material from the solid protein nor from the protein hydrolysates in acid or basic solution. Likewise, dialysis experiments failed to remove the fluorescent material from protein solutions but after hydrolysis with strong acid the fluorescent material is readily removed from proteins by dialysis.

Hydrolysis of proteins by proteolytic enzymes or alkali produced only a slight increase in the amount of fluorescence. However, hydrolysis with hydrochloric acid, sulfuric acid, perchloric acid or phosphoric acid produced large increases in the fluorescence of those proteins containing tryptophane and only slightly increased fluorescence in those proteins which are deficient in this amino acid. The color of the fluorescence produced during acid hydrolysis is blue-green.

Crude commercial proteins produce the same amount of fluorescence, during acid hydrolysis, as these same proteins prepared in a purified state. The presence or absence of air during acid hydrolysis does not affect the amount or color of fluorescence of the protein hydrolysates.

Nineteen amino acids were examined in ultraviolet light for fluorescence. These amino acids were not noticeably fluorescent in the solid state nor in acid, basic or neutral solution.

Amino acid additions to proteins during hydrolysis revealed that tryptophane was the only amino acid which increased the amount of fluorescence during acid hydrolysis. Tryptophane did not affect the amount of fluorescence produced during hydrolysis of proteins with alkali.

When proteins such as zein and gelatin, which are deficient in tryptophane, are hydrolyzed with acid in the presence of this amino acid the amount of fluorescence is greatly increased. However, when tryptophane is added to proteins containing this amino acid the fluorescence is only slightly increased during acid hydrolysis.

The addition of the vitamins which are capable of producing blue-fluorescent compounds did not affect the amount or color of fluorescence during acid hydrolysis.

Boiling acetic acid with proteins greatly increased the amount of fluorescence. However, the fluorescent color produced is much more blue than that of protein solutions or hydrolysates. Boiling acetic acid with several of the amino acids caused a blue fluorescence to appear in the solutions. Strong acids do not produce fluorescence of amino acids. This would indicate that the fluorescence increase with acetic acid and protein is due to a different substance than is produced during acid hydrolysis with strong acids.

Tyrosine and tryptophane were the only amino acids of all those studied which gave blue-green fluorescence with glucose when boiled with dilute HCl. The melanin produced by the action of the tyrosinase of potato juice on pure tyrosine solutions was not fluorescent.

Concentration of the fluorescent material was accomplished by adsorption of the material from acid solutions with English fuller's earth (Cenco fuller's earth was a poor adsorbent) and subsequent elution from this adsorbent with ethyl or methyl alcohol-ammonium hydroxide solutions. From its solubility properties the fluorescent material was found to be different from the blue-fluorescent alkaloid harman produced by the mild oxidation of the acetaldehyde-tryptophane complex. Harman when injected intraperitoneally into rats caused paralysis of the hind legs of the animals and slowing of heart action whereas injection of neutralized protein hydrolysate or the adsorbed material from the hydrolysate had no effect.

Lactoflavin, thiochrome, quinine bisulfate and the fluorescent material from protein hydrolysates give broad bands of fluorescent light. The fluorescent spectrum of the material concentrated from casein hydrolysate is in the violet-green region of the spectrum and is very similar to the fluorescent spectrum of quinine bisulfate. The fluorescent spectra of lactoflavin and thiochrome are different from the fluorescent spectrum of protein hydrolysate. The fluorescent spectrum of the material concentrated from casein hydrolysate is in the violet-green region having wavelengths 4100-5300 Å. The fluorescence of protein hydrolysates is excited only by light of wavelengths 3400-3600 Å.

11839 P

Production of Pernicious Anemia-like Syndrome in Rats with Bile Acids.

RAPHAEL ISAACS.

From the Hematology Laboratory, Michael Reese Hospital, Chicago.

Because of the property of bile acids to produce hemolysis of red blood cells, cytolysis of leukocytes, destruction of nerve cells and myelin of nerve fibers, as well as to produce gastric lesions, a group of symptoms present in pernicious anemia, 12 rats were injected subcutaneously from one to 6 times a week with a suspension of glycocholic acid from ox bile. The effective daily dose after trials with weaker suspensions, was 10 mg. Of 12 rats, 3 developed a mild macrocytic, oval red blood cell anemia during the course of 2 months. During this time, one developed dragging of the hind legs, with a clumsy gait, and in 3 months, 2 showed skin ulcerations in regions which had not been used for injections. These symptoms did not appear in a control group, nor in a group injected with liver extract at the same time as the glycocholic acid.

Table I shows the nature of the blood changes.

The enlargement of the red blood cells appeared before any marked

TABLE I.

Day	R.B.C. millions per mm3	Mean corpuscular vol., μ^3	R.B.C., size†
0	8.4	52.0	25-50-25
1*	8.5	52.0	
7	7.9	51.5	
14	8.8	53.0	
21	7.5	53.5	
28	7.8	55.2	
35	7.0	58.8	11-34-55
42	6.2	55.5	
49	4.5	61.0	
56	4.8	60.2	8-18-74
63‡	4.5	59.7	
70	6.5	60.5	

*Suspension of 10 mg of glycocholic acid in water, 6 times a week.
†Measurements less than 6.0 microns; 6.0 microns; larger than 6.0 microns.
‡Liver extract injections begun.

change in the red blood cell count was noted. An increase in the red blood cell count was noted 2 weeks after the subcutaneous injection of liver extract (7 units, once a week, for 2 weeks). The mean corpuscular volume was still elevated at the end of 2 weeks of therapy (reticulocyte increase).

The fact that but 3 of the 12 rats developed changes, suggests the continuation of the experiments with attention to variations in age of the animals, diet, size of dose, solubility of the preparation used, type of bile acid, frequency and amount of injections, state of the blood and bone marrow before injection, and histological condition of the stomach after the appearance of macrocytes (*cf.* ulcer formation in guinea pigs).

The observations suggest the hypothesis that an increase or defect in the metabolism of bile acids in pernicious anemia causes a defect in the secretion of the "intrinsic factor", resulting in the development of a macrocytic anemia, and permitting an increase in hemolysis. The bile acids, acting on the central nervous system, would cause the degenerative changes. Leukopenia would be another result.

From preliminary observations, it has been established that the bile acids are quite definitely increased in the blood in patients with pernicious anemia in relapse, confirming the report of Blankenhorn.[1]

Summary. A pernicious anemia-like syndrome has been produced in individual rats, characterized by oval macrocytosis and neurological changes following the subcutaneous injection of glycocholic acid.

---

[1] Blankenhorn, M. A., *Arch. Int. Med.*, 1917, **19**, 344.

11840 P

Differences Between High and Low Breast Tumor Strains of Mice When Ovariectomized at Birth.*

GEORGE WOOLLEY, ELIZABETH FEKETE AND C. C. LITTLE.

From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

The following differences were noted between 2 high and one low breast tumor strains of mice when ovariectomized at birth. The high breast tumor strains were the Jackson Laboratory dilute brown "JAX" (dba) and "JAX" C3H strains. These 2 strains have a high incidence of mammary tumors both as breeding and as virgin animals. The low tumor strain was the Jackson Laboratory "JAX" C57 black strain. The observations are based on a group of 125 JAX dba, 20 JAX C3H and 30 JAX C57 black mice. The results for each strain as reported in Table I have been uniform, and we believe deserve preliminary presentation, although not all of the above animals are dead up to the present time.

Easily noticed differences concerned the vagina. The vagina of the ovariectomized JAX dba and JAX C3H mice did not present the classic castrate picture but became much more stimulated than might have been expected. With both strains the vagina opened only a few days later than that of non-castrated littermate females. Progress to obvious mucification and to distinct cyclic cornification is much

TABLE I.
Findings for Animals Ovariectomized at Birth and Observed When Beyond One Year of Age.

	High tumor		Low tumor
	JAX dba	JAX C3H	JAX C57
Vaginal orifice	Open	Open	Closed or small pore
Cyclic vaginal cornification	Yes	Yes	No
Uterine stimulation	,,	,,	,,
Mammary gland	Extensive growth, marked development	Extensive growth, marked development	Variable growth, slight development
Mammary adenocarcinoma†	26	2	0
Nodular hyperplasia of adrenal cortex	Extensive	Extensive	Slight
Mating with male	No	No	No

†The dba and C57 Black strains have reached an older average age than the C3H strain at the present time and, therefore, no estimation of the final percentage of tumors can be attempted.

---

* Supported in part by a grant from the Josiah Macy, Jr., Foundation.

slower than with normal females. The vaginas of some of the JAX C57 black females failed entirely to open although observed from birth up to 20 months of age. Usually, however, a small pore appeared and remained more or less open.

The uterus of the JAX dba and JAX C3H mice after remaining small up to about 6 months of age began to enlarge and attained a size as large, and much larger in most cases, than those of virgin females of a comparable age. The uterine glands were enlarged and sometimes cystic. The JAX C57 black uterus was small and similar to that of a pre-puberty female throughout life.

The mammary glands of the high cancer strain females developed extensively and mammary adenocarcinomas appeared in ovariectomized females of both strains. With the low cancer strain some of the mammary glands remained rudimentary and comparable to those of normal male mice, while others, sometimes in the same mouse developed systems with long but slender ducts. No mammary adenocarcinomas were observed. Even so-called pre-cancerous changes were not present although frequent in the glands of the high tumor females.

The adrenal glands of the JAX dba and JAX C3H mice began to show grossly noticeable areas of nodular hyperplasia[1] in a few cells in the outer edge of the adrenal cortex around 3 months of age.† A number of these areas eventually appeared and enlarged until the adrenal was uneven in contour and several times normal size. All animals had abnormal adrenals. In a few cases cortical carcinomas occurred and attained a size of one centimeter or more in diameter. In the JAX C57 black females very small areas of nodular hyperplasia also occurred in the adrenal cortex and in one case, at least, at around 3 months of age. Few of these areas appeared, however, and but little enlargement of them occurred in later life. The contour and size of the adrenal gland remained quite normal.

Males were experimentally placed with the females but so far no vaginal plugs have been found indicating that normal mating reactions were not attained.

Although the above differences were first noted with mice ovariectomized at birth, they have no necessary connection with early castration in itself. Marked similar strain differences appeared when the ovaries were removed at one month and at 6 months of age.

---

[1] Woolley, G. W., Fekete, Elizabeth, and Little, C. C., *Proc. Nat. Acad. Sci.*, 1939, **25**, 277.

† Some of the early stages were found by William Daughaday, a holder of a Harvard University National Scholarship and a summer student at the Roscoe B. Jackson Memorial Laboratory.

Conclusion. Marked differences appeared in strains of mice when they were ovariectomized at birth and then examined in later life. The high tumor JAX dba and JAX C3H strains showed stimulated uterus, vagina and mammary glands. The adrenal glands exhibited extensive nodular, hyperplastic areas. All of these organs of the low tumor JAX C57 black strain remained essentially unstimulated. To ascertain whether the above differences are characteristic of all high and low tumor strains, other strains are being studied.

11841

Observations on Sensitization of Guinea Pigs with Alum-Precipitated Ragweed Extract.

E. J. Coulson and Henry Stevens. (Introduced by A. K. Balls.)

From the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

Alum-precipitated ragweed pollen extract is a potent antigen for producing anaphylactic sensitization in guinea pigs,[1-4] whereas the watery extract is by comparison a poor one. Alum-precipitated extracts have been successfully applied in sensitizing guinea pigs to house dust[5-8] and cotton linters[5, 6, 9] and in sensitizing dogs to horse serum.[10] The enhanced antigenic response of the alum-precipitated extract is generally attributed to two factors: first, retention of the antigenic substance by the precipitate so that it is only slowly liberated into the system, thus producing a continuous stimulation for the production of antibodies; and second, the local tissue response to the precipitate at the site of injection is said to delay absorption of the antigen. Thus diphtheria toxoid has been detected at the site of injection from a few days to several weeks after administration

---

[1] Harrison, W. T., *U. S. Public Health Reports*, 1934, **49**, 462.

[2] Caulfeild, A. H. W., Brown, M. H., and Waters, E. T., *J. Allergy*, 1936, **7**, 451.

[3] Zoss, A. R., Koch, C. A., and Hirose, R. S., *J. Allergy*, 1937, **8**, 329.

[4] Sherman, W. B., Stull, A., and Hampton, S. F., *J. Immunol.*, 1939, **36**, 447.

[5] Coulson, E. J., and Stevens, H., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 457.

[6] Coulson, E. J., and Stevens, H., *J. Allergy*, 1940, **11**, 537.

[7] Friedman, H. J., *J. Allergy*, 1939, **10**, 479.

[8] Hampton, S. F., and Stull, A., *J. Allergy*, 1940, **11**, 109.

[9] Cohen, M. B., Cohen, S., and Hawver, K., *J. Allergy*, 1939, **10**, 561.

[10] Waters, E. T., Markowitz, J., and Jaques, L. B., *Science*, 1938, **87**, 582.

of alum-precipitated toxoid.[11, 12] The experiments recorded here were designed to determine whether antigenic constituents of ragweed pollen could be detected in the indurated nodule that forms at the site of injection of the alum precipitate in guinea pigs and persists for several months.

Experimental. An alum-precipitated ragweed pollen extract (1-20 dilution) was prepared in the manner previously described.[6] For sensitizing, 1 ml of the whole alum-treated extract was employed for subcutaneous injection because it was found that the antigenic constituents were not completely precipitated by the alum. Thus precipitates, separated by centrifugation from two 1 ml aliquots of the alum suspension, were suspended in 0.9% sodium chloride and injected subcutaneously into 2 normal guinea pigs. The supernatant solutions, without further treatment, were likewise injected into 2 normal animals. Thirty-four days later each animal received an intravenous injection of 1-100 ragweed extract in 0.9% sodium chloride. The results are shown in Table I.

Each animal sensitized with the alum precipitate was found to be fatally sensitized. At autopsy indurated nodules were found at the site of the sensitizing injection. The animals that had been sensitized with the supernatant solution from the alum-precipitated extract showed symptoms of anaphylaxis but were not fatally sensitized. No nodule was present in either of these 2 animals at the site of the sensitizing injection.

After an incubation period of 30 days guinea pigs sensitized with the whole alum-treated extract regularly respond with anaphylactic shock upon the intravenous administration of ragweed pollen extract. Fourteen animals sensitized in this manner were injected intravenously with 0.1 ml of a 1-100 water extract of pollen. Eight responded with immediate fatal anaphylaxis, 4 with severe anaphylaxis, and 2 with moderate anaphylaxis. Of 29 animals sensitized in

TABLE I.
Sensitization with the Precipitate and Supernatant Solution from Alum-Treated Ragweed Pollen Extract.*

Animal No.	Sensitized with	Test injection,† ml	Symptoms
407	Precipitate	0.2	Fatal anaphylaxis, 3½ min.
409	”	0.2	” ” 4 ”
406	Supernatant	0.2	Severe anaphylaxis
408	”	0.4	Slight ”

*Period between sensitization and shock was 34 days.
†Shock material was 1-100 aqueous pollen extract.

---

[11] Glenny, A. T., Buttle, G. A. H., and Stevens, M. F., *J. Path. and Bact.*, 1931, **34**, 267.

[12] Harrison, W. T., *Am. J. Pub. Health*, 1935, **35**, 298.

the same manner and injected with 0.05-0.1 ml of a 1-20 water extract of pollen 23 responded with fatal anaphylaxis and 6 with severe anaphylaxis.

To determine whether antigenic components of ragweed pollen remain unabsorbed in the indurated nodule that appears at the site of injection of the alum precipitate, the following experiments were performed.

Ten guinea pigs weighing between 261 and 347 g were sensitized with alum-precipitated ragweed extract. Thirty-three days later when 2 of the animals were tested for sensitivity by intravenous injections of 0.2 ml of 1-100 pollen extract both responded with fatal anaphylaxis in 2½ and 3 minutes, respectively. The remaining 8 animals were killed by a blow at the base of the skull and the nodules resulting from the sensitizing injection were dissected. Nodules from 2 or 3 sensitive animals were pooled, triturated in 0.9% sodium chloride, and injected subcutaneously into 6 normal guinea pigs. In order to exclude the possibility of passive sensitization 2 of the animals were tested for sensitivity 4 days after injection with the nodules (Table II). Neither of these showed any symptoms of anaphylaxis. Both responded with fatal shock upon injection of pollen extract 28 days later. The 4 remaining animals at the end of the 32-day incubation period were all demonstrably sensitive to the ragweed extract. At autopsy nodules were noted in some of these animals but had disappeared in others.

Discussion. The fact that it is possible to actively sensitize a second guinea pig by the material that is walled in by the cyst which forms at the site of injection of alum-precipitated ragweed extract indicates that the original antigenic substance must be present in practically an unchanged condition and that alum considerably delays the absorption. The continuous stimulus exerted by the slowly absorbed antigen therefore may account for the enhanced antigenic potency of the pollen extract.

TABLE II.
Sensitization with Indurated Nodules.

Animal No.	Sensitized with nodules from animals No.	Incubation period, days	Test injection,* ml	Symptoms
866	760, 788, 789	4	0.2	None
		32	0.2	Fatal anaphylaxis, 3½ min.
870	798, 802, 803	4	0.3	None
		32	0.2	Fatal ” 3½ min.
867	760, 788, 789	32	0.2	Slight ”
868	791, 792	32	0.2	Moderate ”
869	791, 792	32	0.2	Fatal ” 3½ min.
873	798, 802, 803	32	0.2	Fatal ” 6 ”
888	Control		0.5	None

*Shock material was 1-20 aqueous pollen extract.

11842 P

Variation in Immune Response to *Brucella abortus* Depending on Route of Administration.

Paul O. Hageman and J. A. Doubly. (Introduced by David P. Barr.)

From the Department of Medicine, Washington University School of Medicine, St. Louis.

A perplexing diagnostic problem is presented by the combination of vague complaints, low titer *Brucella* agglutination, and positive Brucellergen skin test. Whether such findings indicate past or present infection or whether they lack significance is a fundamental problem.

It was thought that the ingestion of milk containing *Brucella* organisms killed by pasteurization might be responsible for this confusion by stimulating the formation of agglutinins. This hypothesis seemed especially plausible, since heat-killed *B. typhosus* given orally and intravenously yields similar immunological responses, and particularly because of the similarity in the accepted pathogenicities of typhoid and undulant fevers. The investigation of the immune reactions to *Brucella abortus* following injections by these routes was undertaken.

Technic. A stock culture of *Brucella abortus* (National Institute of Health strain No. 456) was grown in 1% dextrose beef infusion broth aerobically at 37°C for 48 hours. The flasks were then heated in a waterbath for 30 minutes at 60°C. Samples of the heated culture showed no growth after 4 days' incubation at 37°C.

Part A. Three groups of 4 rabbits each were used:

Group I received the heat-killed organisms suspended in milk by stomach tube. After centrifuging the heated culture for 20 minutes at 3,000 r.p.m., discarding the supernatant fluid, the organisms were resuspended in the same volume of fresh sweet pasteurized whole milk.

Group II received the heated broth culture intravenously.

Group III served as controls, received no injections, were kept on a regular diet and bled at the same intervals as Groups I and II.

During the first week 0.5 cc of culture or its milk suspension equivalent was given for 3 successive days, in the second week 1 cc was given on 3 successive days, and so on, 1.5 cc in the third week, and 2 cc in the fourth week. Blood was drawn for agglutination tests with the homologous strain before the first dose of organisms was

TABLE I.
Agglutination Titers.

	Rabbit Group	Days 1	Injections begun	7	14	21	31
Part A	I	0*	oral	0	0	0	0
	II	0	intravenous	1:1040†	1:5120	1:5120	1:4480
	III	0	--	0	0	0	0
Part B	I	0	oral‡	0§	0	0	0
	II	0	intravenous	1:640	1:1280	1:1280	1:1280

*0 = No agglutination in dilution of 1:20.
†Figures quoted are averages for the group.
‡Oral dosage of organisms in Part B is 20 × that in Part A.
§One rabbit in this group died of a milk pneumonia because the injection was given intrabronchially. Titer of 1:20 was present just before death.

given, and at weekly intervals thereafter, and again 10 days after the final dose had been given.

Part B. A second batch of heat-killed broth culture of *Brucella* was prepared as described above. Two groups of 4 rabbits each were used, one receiving by stomach tube the same volumes of a milk suspension of the heat-killed *Brucella* culture concentrated 20 times (*i.e.,* 0.5 cc milk contained the organisms from 10 cc of the original culture), and the other receiving the heated culture intravenously in the same doses employed in Part A. Bleedings were made at the same intervals as in Part A.

Results. See Table I.

Part A. Rabbits receiving heat-killed *Brucella* organisms by vein developed agglutinins for that organism, while the same number of organisms given orally failed to stimulate the formation of agglutinin response.

Part B. Increasing by 20 times the number of organisms given orally failed to stimulate formation of agglutinins.

Discussion. Data presented here clearly indicate that, under the conditions of this experiment, heat-killed *Brucella* organisms introduced into the intestinal tracts of rabbits do not lead to the formation of agglutinins. The antigen is either not absorbed or is altered sufficiently by digestive processes so that it loses its antigenicity. In view of these results, ingestion of heat-killed *Brucella* organisms would not seem important as a cause for low agglutination titers for this organism. Likewise attempts to immunize against this infection by oral administration of non-viable *Brucella* organisms would seem futile.

Summary. Rabbits develop agglutinins for *Brucella abortus* following intravenous injections but fail to do so after sizeable doses are introduced into the stomach.

11843

Calcium in Acute Pancreatic Necrosis.*

HUGH A. EDMONDSON AND IRVING A. FIELDS. (Introduced by E. M. Hall.)

From the Department of Pathology, University of Southern California School of Medicine, and the Laboratory of the Los Angeles County Hospital.

In acute pancreatic necrosis neutral fat is split into fatty acid and glycerol by the action of lipase. The glycerol is absorbed and the fatty acids combine with calcium to form soaps.[1]

Recently one of us (H.A.E.) performed an autopsy on a patient who died with symptoms of shock and tetany. Extensive fat necrosis in and around the pancreas and fat embolism were observed. The carbon-dioxide combining power was 47 volumes %, so alkalosis could not have caused the tetany. Serum calcium was not determined antemortem. It occurred to us that possibly due to excessive formation of fatty acids and subsequently calcium soaps, enough calcium had been removed from the blood to cause tetany. On analysis a 2 g sample of pancreas and peripancreatic tissue contained 10.5 mg calcium or 525 mg per hundred g tissue. As there were at least 300 g of tissue so affected some 1500 mg of calcium may be assumed to have been present. This figure is slightly in excess of the total normal amount of calcium in the blood serum and the extracellular fluid combined. The total serum calcium may be estimated at 600 mg. There is about 3 times as much extracellular fluid as blood plasma.[2] Studies of transudates reveal an average calcium content of 7.5 mg[3] or an estimated total of 750 mg in the extracellular fluid.

No published accounts could be found of the total calcium in the lesions of acute pancreatic necrosis or of the serum calcium level in this diease.

We have since studied 2 additional cases which came to necropsy, in one (G.C.) the disease followed surgery and was of less than 24 hours' duration. In this case the entire pancreas and involved peripancreatic tissue was used for analysis. In the second (C.L.) all the tissue (2250 g) was ground 3 times and 3 separate samples were taken which contained almost identical amounts of calcium.

---

* Aided by a gift from Ethel Mossman Jacobs.

[1] Langerhans, R., *Virchows Arch. f. path. Anat.*, 1890, **122**, 252.

[2] Gamble, James L., *Bull. Johns Hopkins Hosp.*, 1937, **61**, 151.

[3] Green, C. H., Bollman, J. L., Keith, M. M., and Wakefield, E. G., *J. Biol. Chem.*, 1931, **91**, 203.

TABLE I.
Calcium Content of Lesions in Acute Pancreatic Necrosis Compared with Normal Human Controls.

	Pancreas. Ca mg per 100 g wet tissue	Peripancreatic fatty tissue. Ca mg per 100 g wet tissue	Total Ca mg in pancreas plus 2 kg of adjacent fatty tissue	
C.R. (control)	5.6	2.6	Estimated maximum	58.0
E.Q. ”	5.3	2.3	” ”	52.0
H.P. ”	6.0	3.6	” ”	78.0
F.H.	525.0		”	1500.0
G.C.	—	—		200.0*
C.L.	178.0	152.0		1732.0

*Disease of less than 24 hr duration.

TABLE II.
Serum Calcium in Eleven Cases of Acute Pancreatic Necrosis (Clinical).

	Serum calcium per hundred cc of blood. Results tabulated on days which represent time elapsed since onset of illness													
Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
B.W			7											
O.B.						8			9.4			9.4		
T.R.	9.6													
V.C.			8.3					9.7						
J.E.			8.7			8.4								
W.D.	10.3													
J.B.							7.9	7.7		7.4	7.6			13.4
								7.7			8.1		10.1	
L.K.	9.5		9.1		9.4									
			8.5	8.5										
M.E.			9.7	9.7										
				9.4										
A.D.				9.7										
J.M.		9		8.9	8.8									

Three normal controls were also studied. The results are given in Table I. To give comparable figures for the controls an estimate was made of the amount of calcium in the pancreas (estimated weight 100 g) plus that in 2 kg of adjacent fatty tissue.

We have also studied the serum calcium (Table II) in 11 individuals who clinically were suffering from acute pancreatitis. In all of these the urinary diastase and/or blood amylase were greatly increased.

Conclusions. As much as 1732 mg of calcium may be present in the lesions of acute pancreatic necrosis. A moderate fall in serum calcium levels in acute pancreatic necrosis may occur between the 3rd and 11th days of illness.

11844

Further Studies on Active Milk Influence in Breast Cancer Production in Mice.*

JOHN J. BITTNER. (Introduced by C. C. Little.)

From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

The purpose of this article is to summarize the published evidence and to present new material that the milk influence in inherited breast tumor production in mice is an actual and active influence.

In this paper the term tumor or cancer signifies breast carcinoma and unless otherwise stated, all the females were used as breeders.

Various experiments have been reported by the author[1-16] on the rôle played by the milk influence, genetic susceptibility and/or hormonal stimulation in the etiology of inherited breast cancer in mice. Others have confirmed the work regarding the milk influence in different strains.[17-20]

The analysis of the data also indicated that another type of breast carcinoma may develop in mice which apparently is not genetic since the progeny do not show tumors. The milk influence is generally inactive in such animals and the other etiological factors are not clear. No difference has been noted in the histological structure of these tumors.

---

* Assisted by grants from the National Cancer Institute and the International Cancer Research Foundation.

[1] Bittner, J. J., *Science*, 1936, **84**, 161.

[2] Bittner, J. J., *Am. J. Cancer*, 1937, **30**, 530.

[3] Bittner, J. J., *Am. J. Clin. Path.*, 1937, **7**, 430.

[4] Bittner, J. J., and Little, C. C., *J. Hered.*, 1937, **28**, 117.

[5] Bittner, J. J., *J. Hered.*, 1937, **28**, 363.

[6] Bittner, J. J., *Am. J. Cancer*, 1937, **35**, 90.

[7] Bittner, J. J., *Am. J. Cancer*, 1939, **36**, 44.

[8] Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 1113.

[9] Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 1590.

[10] Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 1642.

[11] Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 1827.

[12] Bittner, J. J., *Am. J. Cancer*, 1940, **38**, 95.

[13] Bittner, J. J., *Am. J. Cancer*, 1940, **39**, 104.

[14] Bittner, J. J., *Bull. Minn. Med. Foundation*, June, 1940, p. 94.

[15] Bittner, J. J., Third Internat. Cancer Congress, 1939.

[16] Bittner, J. J., *Nat. Cancer Inst. J.*, in press.

[17] Andervont, H. B., and McEleney, W. J., *Pub. Health Rep.*, 1939, **53**, 777.

[18] Andervont, H. B., and McEleney, W. J., *Pub. Health Rep.*, 1939, **54**, 1597.

[19] Korteweg, R., Third Internat. Cancer Congress, 1939.

[20] Snell, G. D., Third Internat. Cancer Congress, 1939.

In the earlier papers it was stated that the incidence of breast tumor in mice ("A" stock) might be reduced from 90% (over 500 animals) to 8% (127 mice) by fostering the young of high breast tumor stock mothers to females of low breast tumor strains. The young were transferred before they were 24 hours old, and only one generation was fostered.

However, some of the young which were permitted to remain with their potentially tumorous mothers for less than 24 hours developed tumors as did some of their progeny. Other mice of the same fostered litter did not develop tumors and had descendants which likewise gave a low incidence of breast tumors, the incidence being similar to that observed in virgin females of the stock.

Fifteen young which were fostered not before from 1-5 days after birth gave a tumor incidence of 87%.

Twenty-seven young of fostered "non-breast cancerous" females were given to control (high cancer) "A" stock females the day of birth; tumors resulted in 89% of these mice. The fostered "non-breast cancerous" females nursed 29 of their progeny and no tumors were recorded.[10]

To determine if the milk influence was present only in the first milk secreted by a lactating female, some high tumor stock females were permitted to nurse their litters for 4 days before being used as foster mothers.[6, 9] The young fostered were BAF_1 hybrids (C_{57} black or "B" ♀♀ x "A" ♂♂). When the high tumor stock females were used as foster mothers the same day their own litters were born, the fostered F_1 females had a tumor incidence of 87.3% (63 animals). Females used one day after their litters were born nursed 54 BAF_1 mice and these females showed a breast tumor incidence of 85.2%. Two mice in each group developed internal tumors and if they are not included the respective ratios were 90.2% and 88.5%. Twelve BAF_1 females were given to "A" stock females which had nursed for 4 days; all of the hybrids developed breast tumors.

Hybrid females (BAF_1) which had maternal parents from a low breast cancer stock and paternal parents from a high breast cancer stock have a spontaneous breast tumor incidence of 2% if they are nursed by their maternal parents.[13, 16] The number of mice observed was 118. If inoculated with grafts of spleen or thymus from 4-week-old "A" stock donors, or lactating mammary tissue from older animals, the observed incidence was 21.7% for 46 mice. BAF_2 control breeding females (246 animals) had a tumor incidence of 0.6% and 11 inoculated mice of the same generation gave 18.2% cancer. Seventeen BAF_2 mice descended from inoculated BAF_1 females showed a mammary gland tumor incidence of

29.4%.[6, 11, 16] The mice of the inoculated groups were older at the time of tumor development than the control animals.

ABF_1 ("A" ♀♀ x "B" ♀♀) females, totaling 122, nursed by "A" stock females showed an incidence of 94.3%, whereas hybrids which had fostered "A" stock (low tumor)† maternal parents gave 0.6% cancer. Another group of 37 ABF_1 females was taken from their high tumor stock mothers and given to inbred C_{57} black stock females with a resulting breast tumor incidence of 8.1% for 37 individuals. Similar mice nursed by "B" stock females which in turn had been suckled by low tumor BZF_1 by B♀ hybrids (2.6% cancer, 39 mice) gave 4.2% cancer for 48 mice. If "B" stock females which had been nursed by high tumor hybrids (BZF_1 by Z or C_3H♀ —79.1% cancer for 43 animals) were used as foster mothers for ABF_1 females, these mice developed tumors in 78.4% of the total or 51 animals.

C_{57} black stock mice when nursed by high tumor stock females ("A" or C_3H) have a breast tumor incidence of approximately 10%.[15, 17] The number tested was 104.

Ten mice of a sub-line of "A" stock fostered mice, having a tumor incidence of 4.5% for 245 breeding females, were fed an average of 5 cc of macerated liver per animal when they were 4 weeks of age. Tumors have not developed and the living animals are 16 months old. Ten additional females received by mouth an average of 1.7 cc of milk obtained from lactating females ("A" stock) 11 to 19 days after their litters were born. One animal died at 356 days of age, 8 have developed tumors, average age 276 days, and the remaining animal is living at 16 months of age. Ten other females, also 4 weeks of age, consumed an average of 0.9 cc of milk from the same source. Seven of the group are living (16 months) and 3 have developed tumors, average age 303 days. This work is being repeated and additional animals are under observation. The "A" stock donors (milk and liver) were from 92 to 155 days of age.

The injection of estrogenic hormones has failed to produce breast tumors in control C_{57} black stock mice.[21, 22] Males of this strain nursed by females of the Paris R III high breast tumor stock will give rise to breast tumor following the implantation of crystals of estrone (Twombly[22]). Paris R III males nursed by C_{57} black stock females had a lower incidence of induced tumors than did the control animals.

---

† Made by fostering "A" stock young to "B" stock females. The use of such animals eliminated the possibility of the hybrid young from obtaining milk containing the active influence.

[21] Suntzeff, V., Burns, C. L., Moskop, M., and Loeb, L., *Am. J. Cancer*, 1936, **27**, 229.

[22] Twombly, G. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 617.

Pellets of theelin (average weight 0.2 mg) and estradiol benzoate (average weight 0.15 mg) were implanted subcutaneously into 6 males and 13 females of the "A" high tumor and 19 males and 24 females of the fostered "A" low tumor stock. Animals dying from too much hormone before the appearance of the earliest tumor, 147 days following injection, are not included. The results for the 2 hormones are combined as they were very similar. Six high tumor females and 3 high tumor males have developed tumors (47.4% of the total). The average tumor age for the females was 189 days and for the males 185 days following injection.

Eleven fostered "A" stock females and 12 males are still alive, all being over 330 days of age. The other females survived an average of 242 days and the deceased males 248 days. No tumors were observed in either group.

Three induced tumors have been noticed among 12 high tumor C_3H stock males and females whereas 18 fostered C_3H animals have been negative. Hybrids have also been injected with estrogenic hormones but many are still living. Tumors have appeared only in the animals which were nursed by high breast tumor stock females.

Discussion. The foster-nursing experiments were started in an attempt to explain the maternal influence observed in breast tumor development in mice.[23]

Since 1936[1] it has been apparent that the incidence of breast tumors observed in high cancer strains of inbred mice might be reduced as the result of foster-nursing the young of such mothers by females of a low breast tumor strain. To account for these results it was assumed that a "breast cancer-producing influence" was transferred through the milk of breast cancer stock mothers to their progeny. Others stated, however, that the absence of an active influence from the milk of cancerous strain females would produce similar results. The importance of the inherited breast cancer susceptibility factor and hormonal stimulation of the mammary tissue were emphasized in later work[8, 9] but need not be considered in detail here.

It was noted in the first experiments that if the fostered progeny of cancerous stock mothers were non-breast cancerous, few tumors were observed in their progeny and descendants. The progeny of "fostered non-cancerous" females nursed by cancerous stock females behaved as control or cancer stock individuals. If the fostered females of a cancerous stock were themselves cancerous, breast tumors were common among their progeny.

---

[23] Staff, Jackson Memorial Laboratory, *Science*, 1933, **78**, 465.

The importance of the time interval between birth and fostering was obvious when tumors were observed in the progeny of cancer stock mothers which were permitted to remain with their maternal parent longer than 24 hours. The incidence for young fostered within 24 hours after birth was low; the incidence for young fostered after 24 hours following birth approached the incidence recorded for the control animals. That the influence was active after the first milk had been secreted was manifested by the use of foster mothers of a cancer strain after they had nursed their own litters for 4 days.

Breast tumors have also resulted following the inoculation of lactating mammary gland tissue, spleen and thymus from donors of a high tumor stock and by feeding 4-week-old mice a small amount of milk obtained from lactating females 11 to 19 days after their litters were born. The incidence and average tumor age seemed to be determined by the amount of the active influence obtained by the animals. Macerated liver fed by mouth has failed to produce tumors although liver obtained from young donors has not been used.

Females of a low breast cancer stock when nursed by high tumor stock females ("A" or C_3H) have a breast tumor incidence of approximately 10%.[15, 17] The incidence is low, presumably, because they do not have the genetic breast cancer constitution. Fostered low breast tumor stock females do obtain the active milk influence when nursed by high tumor females and although they may not develop tumors, the influence may be transferred by nursing to other animals and tumors generally will develop in mice having the genetic breast cancer susceptibility.

The discussion of the relationship of the milk influence to induced estrogenic tumor induction must be delayed until further experimental data are available.

Thus, all the evidence indicates an actual active influence in the milk of cancerous strain females. It is probably present during the entire lactation period as it is active in the first milk, in the milk obtained by nursing 4 days after the birth of a litter and in that secured 11 to 19 days after the beginning of lactation.

Conclusions. The "breast cancer producing influence" is an actual active "influence" present in the milk of high cancer stock females. It is probably present and active during the entire lactation period.

The active influence may be transferred by the inoculation of spleen, thymus, and lactating mammary gland tissue from cancerous stock animals. The active influence probably is not present (or has been destroyed) in the liver of high tumor stock mice. The active influence may be given to 4-week-old females by feeding-by-mouth milk obtained from lactating females of a cancerous stock. Fostered females of low breast tumor strains need not develop breast tumors to transfer the active milk influence by nursing. An active milk influence may be necessary for the development of induced estrogenic breast tumors.

(A future publication will show that a low breast tumor strain of mice may "acquire" an active milk influence at any time, resulting in a high breast tumor strain.)

11845

Appearance of the Melanophore-Expanding Hormone of Pituitary Gland in Developing Chick Embryo.*

GRAHAM CHEN, FRANCES K. OLDHAM AND E. M. K. GEILING.
From the Departments of Pharmacology and Anatomy, University of Chicago.

The purpose of this investigation was to determine when the presence of the melanophore-dispersing hormone can first be demonstrated in the developing chick embryo with a view to correlating the appearance of the hormone with the structural development of the pituitary gland.

Material and Methods. Hybrid eggs (Red-Rock) were incubated for periods of from one to 13 days. In embryos of 5 days and over, the pituitary region was removed with the aid of a dissecting microscope. In the 2-, 3-, and 4-day embryos, the head of the embryo was utilized, and at the one-day stage the entire embryo was used. The material was ground up in a small volume of saline or .25% acetic acid together with a few drops of 2.5% $NaHCO_3$ to bring the material to about pH 8. The resulting suspension was injected into the ventral sac of an hypophysectomized frog. The hypophyseal region of 6 to 20 chicks was used, depending on the age of the embryo.

Certain technical precautions were observed. Only frogs which had been hypophysectomized for at least 2 weeks were used, and the sensitivity of these was insured by testing them with minute doses of standard pituitary extract. Control injections of the acetic-bicarbonate mixture were made prior to injecting the suspension. Further control experiments were made with other tissues or regions of the embryo, and with extracts of acetone-desiccated powders, prepared from both the yolk and white of fertile and unfertile eggs. In no control case was a positive result obtained.

---

* This investigation was supported by grant from the Committee on Research in Endocrinology, National Research Council.

For histological study, serial sections of the pituitary and surrounding structures of chick embryos ranging from 2 to 21 days' incubation were available.

Observation. Melanophore-dispersing activity could be elicited from the hypophyseal tissue of embryos of 5 days' incubation and in all subsequent stages. No attempt was made to estimate the exact amount present. However, it was found that in the 5-day-old embryos an extract prepared from 18 specimens gave a gross darkening of the frog which lasted for about 2 hours, while 9 and 6 specimens respectively gave a slight and a doubtful microscopic expansion of the melanophores.

Examination of serial sections of the chick embryo shows that the hypophysis undergoes considerable structural change between

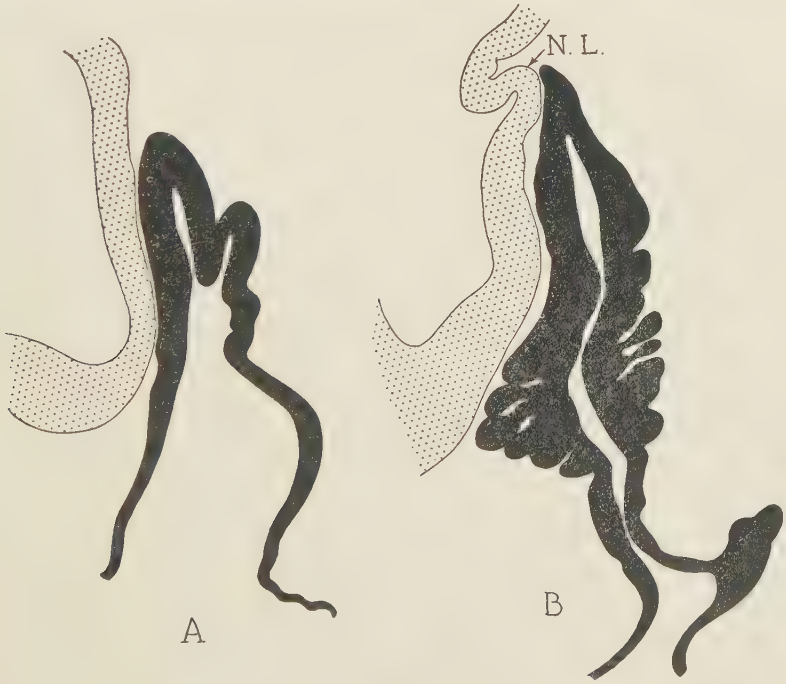


FIG. 1.
A and B. Pituitary gland in chick embryos of 4 and 5 days' incubation respectively. Solid black = anterior lobe. Stippled = brain and neural lobe (N.L.).

the fourth and fifth day of incubation (Fig. 1). In the 4-day embryo the anterior lobe consists essentially of a wide evagination of the buccal epithelium, several ranks of nuclei in thickness. There is no indication of a neural lobe. In the 5-day embryo, the proximal end of the buccal evagination is constricted and attenuated, while the walls of the distal portion have become greatly thickened, with bud-like outgrowths extending into the surrounding mesenchyme. The neural lobe appears for the first time as a small evagination of the infundibulum, which comes in close contact with the tip of the anterior lobe.

Discussion. The hypophyseal extracts prepared as described included varying amounts of neural lobe, brain, and mesenchyme in addition to anterior lobe tissue. However, since in the adult chicken[1] the melanophore-expanding hormone is present in anterior but not in neural lobe extracts, it is assumed that in the chick the hormone arises in the anterior lobe.

The melanophore-dispersing activity in the developing chick hypophysis precedes the appearance of granules in the cells of the anterior lobe. From the above experiments the hormone appears first at the fifth day of incubation, while our own unpublished observations and those of Rahn[2] show that the cells first exhibit a general basophilic tendency at the eighth day and both basophils and acidophils appear at the tenth day. This is in accord with similar observations on the pig embryo. According to Snyder,[3] melanophore-dispersing activity is present in the 30 mm embryo, while Nelson[4] reports the appearance of basophils at 70-100 mm and of acidophils at 160-170 mm, coincidental with the appearance of growth and gonad stimulating activity respectively.

Some investigators, notably Blount,[5] are of the opinion that in at least some species contact with neural tissue is necessary for differentiation and functioning of the intermediate lobe, which elaborates the melanophore-dispersing hormone in those species which possess an intermediate lobe. It may therefore be of significance that melanophore-dispersing activity is not demonstrable in the chick hypophysis until the neural lobe has appeared. Against this, however, is the fact that in the adult chicken the concentration of the melanophore-dispersing hormone is lowest in the juxta-neural region of the anterior lobe;[1, 6] and, furthermore, the changes in the anterior lobe itself are sufficiently marked to account for the appearance of the hormone in the 5-day but not in the 4-day chick embryo.

---

[1] DeLawder, A. M., Tarr, L., and Geiling, E. M. K., *J. Pharm. and Exp. Therap.*, 1934, **51**, 142.

[2] Rahn, H., *J. Morph.*, 1939, **64**, 483.

[3] Snyder, F. F., *Am. J. Anat.*, 1928, **41**, 399.

[4] Nelson, W. O., *Am. J. Anat.*, 1933, **52**, 307.

[5] Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113; *Anat. Rec.*, 1939, **73**, Sup. I, 7.

It is proposed to extend this work to include a study of the appearance of the pressor and oxytocic hormones in the pituitary of the developing chick embryo.

Conclusion. The melanophore-dispersing hormone is first detectable in the developing chick embryo of 5 days' incubation. At this stage, the anterior lobe no longer appears as a simple evagination of the buccal epithelium. The fact that the neural lobe first appears at this stage may or may not be of significance.

11846

A Method of Preparing Isolated Intestinal Loops in the Dog.*

MILDRED E. DOSTER-VIRTUE AND ROBERT W. VIRTUE.
From the Department of Chemistry, University of Denver, Denver, Colorado.

Intestinal absorption experiments were carried on for 2 years with open fistulous loops of the Thiry type as modified by Johnston.[1] The double balloon method as adopted by Riegel[2] was also used for some months. Our results were not satisfactory as leakage often occurred and the animals required too much attention during experimental periods.

Therefore, a new operative method of closing intestinal fistulae has been developed and used during the past 2 years.† Present results indicate it to be successful physiologically, and superior experimentally to former procedures for constructing isolated intestinal loops for absorption studies.

A cylinder of especially prepared bone is implanted at the proximal end of the loop so that granulation tissue invades the interstices of the cancellous bone and forms a sealed closure for the loop.

---

[6] Kleinholz, L. H., and Rahn, H., *Anat. Rec.*, 1940, **76**, 151.

* A portion of this work has been done at the Medical School of the University of Colorado by the courtesy of Dr. R. W. Whitehead.

[1] Johnston, Chas. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 193.

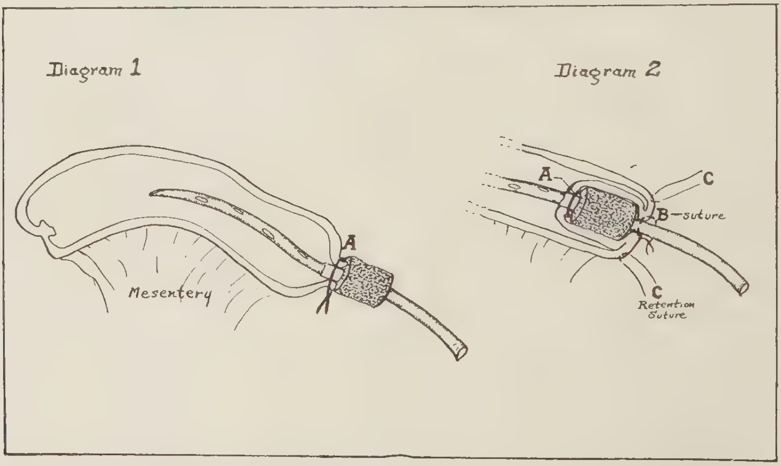
[2] Riegel, Cecelia, Elsom, K. O., and Ravdin, I. S., *Am. J. Physiol.*, 1935, **112**, 669.

† This work was aided by a grant from the New Orleans Academy of Sciences.

Previously reported work with bone transplants has been done by Orell,[3, 4] Bisgard,[5, 6] Albee,[7] Wurm,[8] Girliani,[9] and Pokotilo.[10]

Female dogs varying from 10 to 25 kg in weight have been used for the following surgical procedure. Six to 9 inches of small intestine are isolated for the loop, and the distal end is closed. The intestinal tract is anastomosed by a side-to-side apposition in the smaller animals and an end-to-end in the larger ones, by usual surgical methods. The open end of the loop is then inverted about the cylinder of bone which has been prepared and sterilized with a size 14 catheter through it. The accompanying diagrams show this procedure. Sutures hold the bone cylinder invaginated into the end of the loop so the serosal layer is held snugly to the surface of the bone. The catheter is brought out through the right rectus muscle, and the loop is anchored by retention stitches.

The work of Orell,[11, 12] Ghormley,[13] and Tavernier[14] on heterogeneous bone transplants and implants suggested some methods of



---

[3] Orell, S., *Acta. Chir. Scandinav.*, 1934, **74**, 1.

[4] Orell, S., *Surg. Gynecol. and Obstetrics*, 1934, **59**, 638.

[5] Bisgard, J. D., *Arch. Surg.*, 1935, **30**, 748.

[6] Bisgard, J. D., and Farris, J. M., *Surg., Gynecol. and Obstetrics*, 1938, **66**, 173.

[7] Albee, T. H., *Proc. Royal Soc. Med.*, 1930, **23**, 31.

[8] Wurm, H., *Verhandl. d. deutsch. path. Gesellsch.*, 1930, **25**, 191.

[9] Girliani, G., *Arch. Ital. de Chir.*, 1932, **31**, 268.

[10] Pokotilo, K. L., and Kosdoba, A. S., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1936, **44**, 390.

[11] Orell, S., *J. Bone and Joint Surg.*, 1937, **19**, 873.

[12] Orell, S., *Surg., Gynecol. and Obstetrics*, 1938, **66**, 23.

[13] Ghormley, R. H., and Stuck, W. G., *Arch. Surg.*, 1934, **28**, 742.

[14] Tavernier, *Lyon Chir.*, 1930, **27**, 233.

treating osseous tissue for surgical uses. Our modified procedure is to use the solid areas of femorii or humerii of cattle and cut them into small cylinders about one-half inch long. These pieces of raw bone are placed in 5% alkali overnite, washed with water for one hour, placed in acetone for one hour, and dried thoroughly. Size 14 catheters are cemented into the cylinders by a special rubber cement.‡ The lower end of the bone is also coated with it to seal the porous opening and stop any possible seepage through the cylinder. Ordinary rubber cement and several common plastics disintegrated in from one to 8 weeks in the animal body.

In 10 dogs autopsied or reoperated for removal of the loops, the specimens were studied microscopically. A sealed union of tissue into bone was always present macroscopically. Two microphotographs are presented that demonstrate this invasion of granulation tissue into the scaffolding offered by the bone matrix, and later a definite epithelialization from the serosa into the minute channels of the osseous cylinder. Thus, a combined organic and mechanical

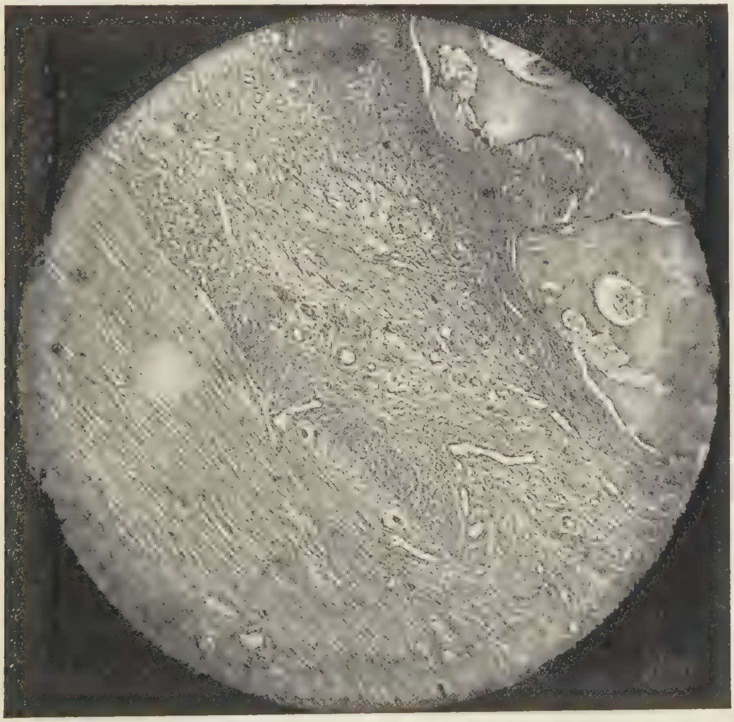


FIG. 1.

Cross section magnification (× 40) of junction of bone and intestinal serosa, 10 days after operation, showing vascular granulation tissue.

---

‡ Gates Rubber Company, No. 8675.

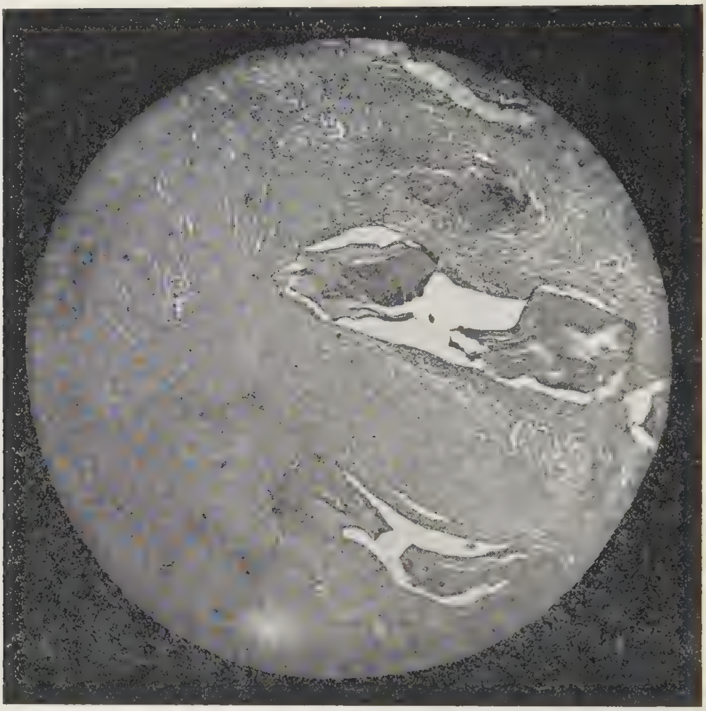


FIG. 2.
Cross section magnification (× 25) of junction of bone and intestinal serosa, one month after operation, with granulation tissue becoming dense and fibrous.

stopper is made at the fistulous openings of these intestinal loops.

About 10 days after operation, experimental absorption or excretion periods are begun. The loop is irrigated daily with tap water, and all materials to be studied are easily introduced by syringes. The ease of working on this type of loop is readily appreciated after one has used the former types of fistulae. As needed, the catheter is closed by a Hoffman clamp, but usually it is left connected to a rubber football bladder that is held beneath the belly by a special metal pan that is strapped to the dog. This closed drainage is a great aid in cleanliness of the animals, and the metal band keeps the dogs from chewing the catheters.

A rubber disc is cemented about the catheter at the skin surface to immobilize it so that over a period of months very little tissue irritation occurs. Experimental absorption studies on these loops will be reported elsewhere.

Thus we present a new operative method of constructing an isolated intestinal loop that allows a convenient, clean, and leakproof closure of the fistula.

11847

Anaerobic Uptake of Potassium by Frog Muscle.

R. B. Dean. (Introduced by W. O. Fenn.)

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Steinbach[1] has shown that frog muscle loses potassium in exchange for sodium when the muscle is soaked in potassium-free Ringer's solution. He also showed that when potassium depleted muscles are placed in Ringer's solution containing potassium, they regain potassium at the expense of sodium. This uptake of potassium in exchange for sodium requires work which must be supplied by the muscle. It has previously been shown that muscle retains its potassium in Ringer's solution in the absence of oxygen.[2] The present investigation was undertaken to determine the influence of aerobic respiration on the uptake of potassium under the conditions of Steinbach's experiments.

The sartorius, semitendinosus, and tibialis anticus longus muscles from recently caught frogs were dissected and placed in potassium-free Ringer's solution containing M/3000 phosphate buffer, pH 7.4. After one hour the muscles from one frog were transferred to 250 ml of K-free Ringer's solution which was stirred by a current of air and kept in a cold room at 4°C. After about 17 hours the muscles were divided into sets for further treatment. This treatment was of 3 sorts, (a) immediate analysis, (b) analysis after immersion for 4 or 7½ hours in 25 ml of Ringer's solution containing 10 mM K and M/150 phosphate buffer and continuously equilibrated with air, (c) the same as (b) but continuously equilibrated with pure nitrogen starting ½ hour before the muscles were introduced. Paired sets of muscles were used to compare treatments (a) and (b), (b) and (c), and (a) and (c), as shown in Table I. Fenn and Cobb[3] state that muscles lose more potassium in Ringer's solution containing m/3000 phosphate than in M/150 phosphate. One incomplete experiment showed no difference in the uptake of potassium from solutions containing M/3000 and M/150 phosphate buffer.

On removal from the solution, the muscles were individually blotted, weighed on a torsion balance, and placed in small weighing bottles. The bottles were dried over night at 100° and the loss in weight was taken as the water content. On small muscles weighing less than 150 mg this procedure is accurate to within 1%. Chloride was determined by a new micro-diffusion method in which HCl gas is liberated from the dry tissue by concentrated H_2SO_4 and absorbed by NaOH on a piece of filter paper fastened to the lid of the weighing bottle. Chloride was determined on the paper by a modification of the method of Van Slyke.[4] The method and its errors will be described elsewhere. Potassium was determined on the sulphuric acid digest by the method of Wilde.[5] In this way determinations of water, chloride, and potassium could be made on the same tissue. The significance of the results was tested by Students' test, taking a probability that the sets are equal of less than 0.05 as significant.

---

[1] Steinbach, H. B., *J. Biol. Chem.*, 1940, **133**, 695.

[2] Dean, R. B., *J. Cell and Comp. Physiol.*, 1940, **15**, 189.

[3] Fenn, W. O., and Cobb, D. M., *J. Gen. Physiol.*, 1934, **17**, 629.

Table I contains values averaged by sets for the results obtained on 10 frogs during the month of August. K_m and Cl_m are reported in micromols per gram of whole muscle. The fiber water was calculated on the assumption that all the chloride is extracellular and at a concentration of 114 millemolar. K_f represents the concentration of potassium in the fiber water. It is calculated from K_m by deducting the potassium in the extracellular phase which is assumed to have a concentration equal to that of the potassium in the Ringer's solution. A fiber potassium concentration, K_c, has also

TABLE I.

Effect of Oxygen and Nitrogen on Intake of Potassium from a High Potassium Solution.

Set	Gas	Hr in 10 mM K	No. of muscles	Dry wt, mg	H_2O %	K_m	Cl_m	Fiber H_2O %	K_f	K_c	K_d
a	control	0	5	39.6	81	59	26	58	**105**	**105**	**302**
b	O_2	7½	5	39.2	81	69	36	49	**137**	**125**	**355**
a	control	0	8	27.8	81	62	33	52	120	120	**326**
c	N_2	7½	8	26.5	83	66	38	49	128	119	**354**
b	O_2	7½	9	20.3	82	63	50	39	**152**	**141**	**329**
c	N_2	7½	9	21.6	83	62	46	43	**135**	**126**	**346**
b	O_2	4	6	25	81	76	33	52	**139**	**128**	383
c	N_2	4	6	25	82	70	35	51	**128**	**118**	377

Average values for small isolated frog muscles. All concentrations are in micromols per gram. K_m and Cl_m are calculated per gram net weight. K_f is concentration in the water of a chloride free fiber. K_c is concentration in the water of chloride permeable fiber. K_d is amount per gram dry weight. Pairs of values in bold face type are significantly different.

---

[4] Van Slyke, D. D., *J. Biol. Chem.*, 1923, **58**, 523.

[5] Wilde, W. S., *J. Biol. Chem.*, 1939, **128**, 309.

been calculated on the assumption that chloride penetrates the fiber and has a concentration in the fiber water equal to the potassium concentration in the Ringer's solution. This assumption follows from the theoretical treatment of Conway and Boyle[6] (see Dean[7]) for fibers that are permeable to chloride as is probably the case when the external postassium is as concentrated as 10 millemolar. A potassium content per gram of dry weight has also been calculated, K_d, allowing for potassium in the extracellular spaces. This was calculated for a chloride-free fiber; values of K_d, calculated on the assumption that the fiber is chloride permeable, are slightly greater in set c. Since the dry weights show no consistent variation between paired sets, they probably are a good indication of the initial weights which were unfortunately not recorded.

The values of K_d, K_c, etc., were calculated individually for each muscle and the results averaged. If the values of these concentrations are calculated from the average values of K_m, Cl_m, and H_2O somewhat different results will be obtained because of the variability of the original data.

On the basis of potassium content K_m, there is no significant difference between the sets. However, the concentration of potassium inside the fibers K_f or K_c, is significantly greater in set b in air than in the initial set a, or the anaerobic set c. There is no significant gain in the concentration of fiber potassium in nitrogen. However, when we consider the amount of potassium in the fiber, K_d, it is apparent that after 7½ hours that the fibers in nitrogen have gained more potassium than either of the other two sets, and after 4 hours the gain is only 2% less in nitrogen than in oxygen. This paradox is explained by the fact that the muscles in nitrogen swell considerably and an increase in fiber size requires an uptake of potassium to keep the concentration constant.

This work shows that muscle fibers can take up potassium against a concentration gradient anaerobically as well as aerobically, and supports the previous work,[2] where it was shown that muscle fibers are permeable to potassium yet maintain their normal potassium concentration in nitrogen just as in oxygen. The fact that the fibers in nitrogen failed to increase their potassium concentration, might be explained by supposing that the fibers were already doing all the work that was possible just to maintain their potassium concentration as the fiber increased in volume.

---

[6] Conway, E. J., and Boyle, P. J., *Nature*, 1939, **144**, 709.

[7] Dean, R. B., *Biol. Symposia*, 1940, in press.

11848

Failure of Thyrotropic Pituitary Hormone to Prevent Spontaneous Mammary Cancer in Mice.

C. D. Haagensen, H. T. Randall and R. Auchincloss.
(Introduced by E. T. Engle.)

From the Surgical Pathology Laboratory, College of Physicians and Surgeons, Columbia University, New York.

Since Lacassagne proved that mammary carcinoma can be induced in mice by means of estrogens, workers in several laboratories have sought to discover what rôle, if any, pituitary hormones play in the development of this disease. The close relationship of ovary and pituitary gland in other biological processes makes this kind of research reasonable.

Cramer and Horning, in the laboratory of the Imperial Cancer Research Fund in London, gave a series of female mice of the RIII strain thyrotropic pituitary hormone subcutaneously, and reported[1] that they were able to prevent the development of mammary carcinoma in this strain by this means. They used a watery solution of pituitary hormone, sold under the trade name of *Ambinon*.* It contained, according to biological assay, 150 guinea pig units of thyrotropic hormone and 50 units of gonadotropic hormone per cubic centimeter. This preparation was injected twice weekly in doses of 0.1 cc into the subcutaneous tissues of the mice. Only 19 mice were used. Their treatment was begun when they were 2 months of age. It was still being continued when Cramer and Horning wrote their report about a year later. At this time none of the mice had developed mammary carcinoma, and the authors felt justified in concluding that the development of the disease had been prevented by the hormone. Although these female mice had been kept with males, none of them had littered during the period of treatment. In a control series of 22 untreated female mice, which although kept with males had borne no litters, Cramer and Horning observed 11, or 50%, develop mammary carcinoma.

We have been able to repeat this experiment on a somewhat larger scale, using the same strain of mice and the same preparation of pituitary hormone. The RIII strain of mice has been bred by brother and sister matings in our laboratory since 1935, when we obtained 6 members of the strain directly from Lacassagne in Paris.

---

[1] Cramer, W., and Horning, E. S., *Lancet*, 1938, **1**, 72.

* Purchased from the N. V. Organon Co., Oss, Holland.

Our control material now includes some hundreds of animals. The pituitary hormone was purchased from the same firm that supplied the London authors. We realized that the preparation was not a very pure one, containing as it did 50 units of gonadotropic hormone in addition to 150 units of thyrotropic hormone per cubic centimeter. Since we were trying to repeat the London authors' experiment we had no choice except to employ it.

We treated a total of 33 mice. Of these 29 survived for at least 6 months, the lower age limit for the development of spontaneous mammary carcinoma in our control series. The treatment was begun when the mice were just under 2 months of age, 0.1 cc of the hormone being given twice weekly. It was continued for fifteen months. At the end of this time 13 mice remained alive. Our supply of hormone being exhausted, and its failure to prevent mammary carcinoma being apparent, the treatment was discontinued. All of the mice in the series are now dead and we are able to present the complete data. The fate of the 29 mice is shown in Table I.

Among these 29 treated mice that survived 6 months, 16 therefore

TABLE I.

Mouse No.	Age (months) treatment began	No. Litters	Age (months) treatment ended	Age (months) death	Mammary carcinoma
801	1.6	0	9.7	9.7	0
803	1.7	2	14.5	14.5	+
805	1.6	0	16.3	27.0	0
807	1.5	3	11.3	11.3	+
809	1.6	0	14.7	18.0	0
815	2.1	1	15.7	16.4	+
817	1.9	0	15.5	25.4	0
819	1.8	0	8.9	8.9	0
821	1.9	1	15.1	15.1	+
823	1.9	0	16.7	31.3	0
825	1.8	0	14.8	19.3	0
827	1.7	0	15.8	15.8	+
829	1.6	0	14.6	16.9	+
831	1.7	0	7.2	7.2	0
833	1.2	0	10.8	10.8	+
835	1.4	2	14.6	29.2	0
837	1.3	2	15.5	15.5	+
841	1.5	0	14.5	17.0	0
843	1.4	1	14.3	19.8	+
847	2.0	1	16.1	26.0	0
849	1.5	0	16.1	20.8	0
851	2.1	3	10.5	10.5	+
853	1.4	3	10.5	10.5	+
855	1.7	2	9.4	9.4	+
857	1.6	0	13.9	24.0	0
859	1.6	2	8.5	8.5	+
861	1.7	2	10.3	10.3	+
863	2.0	1	12.9	12.9	+
865	1.8	0	9.8	9.8	+

developed mammary carcinoma. This tumor incidence is not significantly different from that for our untreated, unbred controls, and slightly lower than that for our untreated bred controls. In the light of our knowledge that breeding increases the incidence of spontaneous mammary carcinoma in this strain of mice this result might be expected, for the breeding record of this group of *Ambinon*-treated mice was intermediate. Only 14 of the 29 bore litters, averaging 1.8 litters each. In our untreated control series of bred females 128 of the 140 that survived 6 months bore litters, averaging 5.5 litters each. Our conclusion must be that the treatment with *Ambinon* prevented, to some extent, pregnancy in our mice, and therefore lowered slightly the incidence of mammary carcinoma. It certainly did not abolish the disease as Cramer and Horning claimed.

Histological study of the adrenals, ovaries, uterus, mammary glands, and mammary carcinomas when they developed, in these treated mice showed no abnormality which we have not found in the tissues of our control animals. In this strain of mice certain changes in the endocrine glands, which might be called degenerative, occur spontaneously. The thyroids were studied with special care, but the only difference between those of the treated animals and the controls that could be made out was a slightly greater cellularity of the glands in some of the former. The pituitary showed no gross changes in the treated mice. We were not able to carry out adequate histological study of this organ.

The statistical data for the treated animals and our controls is given in Table II.

Since 1938, when Cramer and Horning published their report of the carcinoma *inhibiting* effect of anterior pituitary, and we under-

TABLE II.

	Mice treated with thyrotropic hormone	Unbred control mice	Bred control mice
Total number surviving 6 mo.	29	94	140
Developed mammary carcinoma	16 or 55.2% ± 9.23%	49 or 52.2% ± 5.15%	103 or 73.6% ± 3.72%
Mean age at death of those with carcinoma	13 mo. ± .79	16.44 mo. ± .73	12.15 mo. ± .36
Median age at death of those with carcinoma	12.1 " ± .98	16.5 " ± .91	11.3 " ± .45
Standard deviation of age at death of group with carcinoma $\left(\sqrt{\frac{\Sigma d^2F}{N} - b_x}\right)$	3.15 " ± .56	5.12 " ± .52	3.67 " ± .25

Note: All corrections are standard deviations.

took to verify their findings, Loeb and his associates[2] have reported that the same organ, when transplanted subcutaneously, causes the carcinoma rate to be *increased*. We have not attempted any transplantation experiments, but our data concerning the results of subcutaneous injection of the hormone may apply to some extent to Loeb's new interpretation of anterior pituitary function. Cramer[3] has presented an elaborate theory of the genesis of mammary carcinoma in which the pituitary takes an important rôle as an antagonist of the disease. His theory would appear to be premature.

Summary. Long continued subcutaneous injection of an anterior pituitary preparation which contained thyrotropic hormone and some gonadotropic hormone did not definitely affect the incidence of mammary carcinoma in female mice of the RIII strain.

11849 P

Typhus Rickettsia Isolated from Mice and Mouse-Fleas During an Epidemic in Peiping.*

Wei-t'ung Liu and Samuel H. Zia.

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

It is now generally recognized that "endemic" or murine typhus has its reservoir in rats and is transmitted to man by rat-fleas, particularly *Xenopsylla cheopis*.[1, 2] However, in South Queensland. Australia, circumstantial evidence strongly incriminates mice, rather than rats, as the reservoir, although actual proof of infection among these rodents or their ectoparasites is as yet lacking.[3, 4] Lépine and Lorando,[5, 6] in Athens, examining rats as well as mice trapped in the premises where cases of typhus had occurred, found that whereas rats were frequently infected with typhus Rickettsia, mice were uniformly free from the infection. Sparrow,[7] on the other hand, recovered 2 typhus strains from the pooled brain emulsions of 10 out of 300 mice *(Mus musculus gentilis)* caught at random from different households in Tunis. Brigham[8] also obtained a typhus strain from a field-mouse *(Peromyscus polionotus poliomotus)* in the rural district of southern Alabama where cases of typhus had developed under conditions which made the rat a "highly improbable causative factor." It is clear from the above reports that hitherto the association of typhus-infected mice and mouse-fleas with the disease in man has not been conclusively demonstrated, and hence the rôle of mice as another reservoir of "endemic" typhus can not be considered as settled.

---

[2] Loeb, L., and Kirtz, M. M., *Am. J. Cancer*, 1939, **36**, 56.

[3] Cramer, W., *Am. J. Cancer*, 1940, **38**, 463.

[*] We are indebted to Dr. A. B. D. Fortuyn for identification of the species of mice and to Dr. H. F. Hsü for that of fleas.

[1] Biraud, Y., and Deutschman, S., *League of Nations Epidem. Rep.*, 1936, **15**, 99.

[2] Zinsser, H., *Am. J. Hyg.*, 1937, **25**, 430.

[3] Wheatland, F. T., *Med. J. Australia*, 1926, **1**, 261.

[4] Strickland, C., *Far East. Assn. Trop. Med. Trans. Ninth Congress*, 1927, **2**, 517.

[5] Lépine, P., *Compt. rend. Soc. de biol.*, 1934, **117**, 848.

[6] Lépine, P., and Lorando, N., *Bull. Soc. path. exot.*, 1936, **29**, 285.

In March, 1940, a typhus epidemic occurred in a small household in this city, affecting successively 9 out of 10 members of the family. In all of them, the clinical symptomatology was characteristic; 2 were admitted into this hospital and showed, in addition, a positive Weil-Felix reaction.† They admitted that they harbored lice through the greater part of the year and not infrequently encountered fleas in their clothings and beddings. Rats were scarce, but the house was heavily infested with mice. Four mice *(Mus wagneri)* were captured, and 12 fleas collected from these animals were identified as *Leptopsylla musculi.* By inoculating separately the brains of the mice and 10 of the fleas into the peritoneal cavity of 2 guinea pigs, 2 typhus strains were recovered. Each strain was passed through guinea pigs for 11 generations. In most of the animals, the infection was manifested by fever (40.0-41.5°C), lasting from 2 to 9 days, after an incubation period of from 2 to 11 days. In those sacrificed 10 to 14 days after the onset of fever, typhus nodules were either absent or scanty in the brains. In some of the animals, the tunica vaginalis exhibited various degrees of congestion and exudation, but in none was a scrotal swelling observed. After 4 and 5 passages in guinea pigs, the 2 strains were transferred to albino rats and have been carried separately from rat to rat for 10 generations to date. They produced in the rats a definite febrile reaction (38.1-39.5°C), lasting in most cases from 2 to 8 days, after an incubation period of from 4 to 10 days. Some of the animals killed on the 10th to the 18th day of infection gave a positive Weil-Felix reaction at titers varying from 1:10 to 1:640. The brains of rats of the 1st and 8th generations of each series were inoculated into guinea pigs. All of the 4 animals used developed typical fever; the one inoculated intraperitoneally with the brain of a rat of the 8th generation of the flea series showed a moderately pronounced scrotal swelling, and Rickettsiae were grown from the tunica vaginalis in Maitland culture and Zinsser agar tissue media.[9]

---

[7] Sparrow, H., *Arch. Inst. Pasteur,* Tunis, 1935, **24**, 435.

[8] Brigham, G. D., *U. S. Pub. Health Rep.,* 1937, **52**, 659.

† From one of the cases, a third typhus strain was obtained from body lice in the garments, which so far does not seem to differ experimentally in any important respect from the mouse and the flea strains herein reported.

Comment. In 1938, one of us (Zia),[10] examining over 100 mice trapped at random from different households in this city, failed to isolate typhus Rickettsia from any of these animals. The fact that the present strains were recovered from 4 mice and their fleas in a house in which an epidemic had developed naturally suggests a causal relationship between the disease in men and the infection in mice. 1. If the disease in men were historic typhus, the epidemic having started from a human carrier, it would be indeed difficult to conceive, in view of the experimental observations of Nicolle and Giroud[11, 12] how the mice could have acquired the infection from man. 2. We are, therefore, inclined to think that the epidemic was of murine origin, the infection being conveyed accidentally from mice to man by mouse-fleas and thereon, independent of mice and mouse-fleas, from man to man by human body lice. Although the mouse-fleas, *L. musculi,* ordinarily do not attack man, it is not inconceivable that they may do so occasionally, when men live in close association with mice, especially if many of the mice should die in an epizootic, as during the years 1925 and 1928 in South Queensland. Moreover, as the number of mouse-fleas we examined was small, we are not certain that the mice were not infested with some other fleas which may leave their normal host more readily than *L. musculi.* In this connection it is worthy of note that a number of workers have shown that the Rickettsia of murine typhus is present in the urine of infected rats, and have suggested that the disease may be conveyed to man by ingestion of contaminated food without the mediation of fleas.[13–16] 3. It is, of course, possible that the initial case of the epidemic had contracted the disease from rats through rat-fleas, the mice being only secondarily involved in the course of infection among rats, but this does not seem very probable, since mice were greatly preponderant over rats in the house, and from the observations of Lépine and Lorando[5, 6] and Sparrow,[7] it appears that natural transmission of typhus infection from rats to mice in the same households does not commonly occur. It must be said, however, that in general, inasmuch as rats are considerably more flea-infested than mice, and the common rat-flea, *X. cheopis,* attacks man much more readily than the common mouse-flea, *L. musculi,* rats are probably much more important than mice as reservoir of "endemic" typhus.

---

[9] Zinsser, H., FitzPatrick, P., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

[10] Zia, S. H., unpublished observations.

[11] Nicolle, C., and Giroud, P., *Compt. rend. Acad. d. sc.*, 1934, **199**, 1169.

[12] Nicolle, C., and Giroud, P., *Arch. Inst. Pasteur,* Tunis, 1935, **24**, 47.

[13] Marcandier, A., and Pirot, R., *Bull. Soc. path. exot.*, 1933, **26**, 349.

[14] Nicolle, C., Giroud, P., and Sparrow, H., *Arch. Inst. Pasteur,* Tunis, 1934, **23**, 1.

[15] Le Chuiton, F., Berge, C., and Pennanéac'h, J., *Bull. Soc. path. exot.*, 1936, **29**, 831.

[16] Le Chuiton, F., Pirot, R., Berge, C., and Pennanéac'h, J., *Bull. Acad. Med.*, 1938, **119**, 175.

11850

Cultivation of *Clostridium tetani* in Unfertilized and Developing Fertilized Hens' Eggs.

Kuo-ch'ing, Chen. (Introduced by S. H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

The employment of fertilized egg has proven of value for the cultivation of some bacteria and a number of filterable viruses. The chorio-allantoic membrane has been mostly used as the site of infection. Since the cultivation of anaerobic microörganisms in unfertilized and developing fertilized eggs has not been recorded, the preliminary results of such a study with an obligatory anaerobe, *Cl. tetani,* is herewith communicated.

Technic. A pure toxigenic strain of *Cl. tetani,* recently isolated from a patient, was used. Cultures in cooked meat tube after 48 hours' growth were used as the inoculum. Unfertilized and developing fertilized hens' eggs of different age were employed. After the position of the embryo and the air sac was identified by transillumination and marked off and the shell opposite the air sac was sterilized with tincture of iodine, a small opening was made with a carborundum disc attached to a dental drill, leaving the inner shell membrane intact, which was later cut with a small knife after resterilization with 70% alcohol and heat. A loopful of inoculum was then introduced through this opening to the center of the yolk sac, and in the case of fertilized eggs toward a point at some distance from the embryo. The shell aperture was then closed with melted hard paraffin. The eggs were then incubated at 37°C. Different batches of them were taken out and examined grossly and by stained smears microscopically. Aerobic contaminations were excluded in every instance by absence of growth of the eggs' content on blood agar plates.

Results. At first, cultivation was done in unfertilized eggs. Fifty-four eggs were divided into 9 groups of 6 each: One group unheated, others heated in water bath at 90°C for 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0 minutes respectively, and still others fully cooked by boiling for over 15 minutes. Inoculation into the heated eggs was done after they had been cooled. One egg from each group was used for examination at 2nd, 3rd, 4th, 5th, 7th, and 14th day of incubation. From the 7th day, about ⅓ of the eggs heated for from 2-5 minutes began to show some blackening and liquefaction, at the same time a foul odor was present, all of which became progressively worse. All the raw and fully cooked eggs and the remaining ⅔ of the heated eggs showed no apparent change up to 2 weeks of incution and hardly any bacilli were found microscopically. On the other hand, numerous gram-positive bacilli with terminal spores could be seen in the liquefied and blackened eggs. The number of organisms appeared to be in direct proportion to the degree of proteolysis and blackening. The outer layer, consisting of hardened egg-white, remained intact.

It is difficult to determine the optimum length of time required for heating the eggs as the results have not been consistent. Nevertheless, the experiment did suggest the heating must be sufficient to harden the outer layer of the egg-white but must not be too long to effect a complete hardening of the egg-white.

Next, an attempt was made to grow the organisms in developing fertilized eggs of different ages. The incubation of eggs before inoculation was at 38°C. Eggs with viable embryos of 4, 6, 8, 10, and 12 days of age, were seeded with the culture broth. One egg from each group was used for examination at different periods as in the first experiment. It was found that all the eggs examined showed various degrees of proteolysis and blackening on the second day of incubation. Complete liquefaction set in from the third day on. The embryos were invariably dead. Numerous gram-positive bacilli with terminal spores were found in the contents of the eggs. The eggs with 6- to 10-day-old embryo yielded the most abundant growth. The results have been consistent and therefore dependable.

From the above experiments, it can be seen that the developing fertilized eggs serve as a better medium for cultivating *Cl. tetani* than the heated unfertilized eggs. A third experiment was made to investigate whether heating the fertilized eggs would enhance the growth of *Cl. tetani.* Fertilized eggs with 6-7-day-old embryo were divided into 2 groups: one group unheated, the other heated in water bath at 90°C for 3 minutes; and a number of unfertilized eggs similarly heated was included for comparison. The results were somewhat unexpected. Heating of fertilized eggs retards instead of accelerates the growth of *Cl. tetani.* But they still gave earlier and better growth than the heated unfertilized ones.

Comment. In the cultivation of anaerobic microörganisms, it is well known that they are not as fastidious about their medium requirements as they are about the anaerobic environment. The growth of *Cl. tetani* in some of the heated unfertilized eggs may be due to a better anaerobic condition, as heating drives off the air in the egg and the hardened layer of egg-white prevents the re-entry of air from outside.

There may be two reasons for the luxurious growth of *Cl. tetani* in developing fertilized eggs. Firstly, the developing embryo consumes oxygen and thus creates a relatively better anaerobic condition, and, secondly, the presence of fresh animal tissue—the embryo—may stimulate growth. Perhaps this stimulating factor may be destroyed by heating.

The addition of animal tissue to media for the cultivation of anaerobes is not new. Von Hibler's brain medium,[1] and Holman's cooked meat tubes,[2] are notable examples. As pointed out by Holman, although the use of fresh tissue may be necessary for the growths of some of the stricter anaerobes, there is always present the disturbing possibility of contamination, not so much from without as from the animal tissues themselves.[3] The developing chick embryo has the advantage of being a living tissue free from contamination.

---

[1] Hibler, E. V., *Untersuchungen die pathogenon anaeroben,* Fischen, Jena, 1908.

[2] Holman, W. L., *J. Bact.*, 1919, **4**, 149.

[3] a. Theobald Smith, *Cent. f. Bakt.*, 1890, **7**, 502; b. Ford, W. W., *Trans. Assn. Am. Phys.*, 1900, **15**, 389; c. Wolbach, S. B., and Saiki, T., *J. Med. Res.*., 1909, **21**, 267.

11851 P

Experimental Studies upon *B. violaceous,* Isolated from a Fulminating Fatal Human Infection.*

Herbert J. Schattenberg. (Introduced by Charles W. Duval.)
From the Department of Pathology, Tulane University, New Orleans, Louisiana.

Bacillus violaceous (Chromobacterium violaceum) is considered a common saprophytic microörganism occurring in soil, water, sewage and contaminated food. It is generally regarded as non-pathogenic for man and lower animals.

Woolley[1] reported fatal infections of caribao in Manila with this microörganism which resembled hemorrhagic septicemia of cattle. He also established the pathogenicity of the isolated culture but did not find evidence of toxin-production. Recently Black and Shahan[2] and Soule[3] reported instances of human infection with *B. violaceous* which persisted for many months and finally proved fatal. Soule demonstrated pathogenicity for laboratory animals.

The culture employed in the present experiments was isolated in pure state on ordinary media from the lesions present in various organs at the necropsy of a human case. The infection arose from a wound of the foot. There ensued a marked inguinal adenitis, septicemia, as shown by a positive blood culture, and death within a week.

Studies of pathogenicity and animal protection have been performed with this culture.

Pathogenicity Experiments. 12 rabbits, 10 guinea pigs and 12 white mice were employed. Six rabbits received intravenously 1 cc of a saline suspension containing approximately one billion microorganisms per cc. The suspension was made from the growth of *B. violaceous* on nutrient agar. All 6 animals died in approximately 24 hours. The necropsies showed no macroscopical lesions. Cultures of *B. violaceous* were recovered from the lungs, liver, spleen, and heart's blood. The microscopical study of sections revealed degenerative changes, particularly in the liver.

Six rabbits were injected intraäbdominally with a broth culture of

---

* Aided by a grant from the David Trautman Schwartz Research Fund.

[1] Woolley, P. G., *Johns Hopkins Bull.*, 1905, **16**, 89.

[2] Black, M. E., and Shahan, J., *J. A. M. A.*, 1938, **110**, 1270.

[3] Soule, M. H., Abs. Scientific Proceedings, Am. Assn. of Pathologists and Bacteriologists, *Am. J. Path.*, 1939, **15**, 592.

B. violaceous in amounts of .5 cc, 0.75 cc, 1 cc, 1.25 cc, 1.5 cc, and 2 cc. Those receiving 2 cc and 1.5 cc died in 12 hours and 24 hours respectively. *B. violaceous* was grown from heart's blood and visceral organs. The animals injected with 1.25 cc and 1 cc died in 5 days. The lungs, spleen, and liver presented light yellow foci of necrosis varying from pin-point size up to 4 mm. Microscopically the lesions presented necrosis, karyorrhexis and karyolysis with little inflammatory cellular response. These lesions conformed in gross and microscopical appearance to those present in the human case. No violet color was present but *B. violaceous* was readily recovered in pure state from all lesions and also from heart's blood.

Ten guinea pigs were injected intraäbdominally, 5 receiving 0.25, 0.5, 0.75, 1.0, and 1.5 cc of saline suspension of *Bacillus violaceous* from agar slants and contained approximately one billion bacilli per cc. Five other animals received similar injections of broth culture grown for 4 days and diluted to contain approximately one billion bacilli per cc; all died within 16 hours. No discernible lesions were found although *B. violaceous* was recovered from visceral organs and heart's blood. Microscopically, degenerative changes occurred in most parenchymatous structures. Three guinea pigs receiving 0.75, 1.0, and 1.5 cc of the saline suspension died in 24 hours and presented a few minute scattered lesions in the liver. The lungs were greatly congested. Microscopically the hepatic lesions were of a granular, necrotic type showing fragmented nuclei. *B. violaceous* was recovered from the organs and heart's blood. The 2 remaining guinea pigs receiving 0.25 and 0.5 cc have survived for several weeks.

Twelve mice were injected intraäbdominally, 6 with 0.1 cc and 6 with 0.5 cc of suspensions of *B. violaceous* like those employed for the guinea pigs and died within 48 hours. Those receiving the broth culture died more quickly than those injected with the saline suspensions from agar slants. No gross lesions were produced but the microscopical study revealed evidence of toxic degenerative changes particularly in the liver lobules. *B. violaceous* grew readily from the heart's blood, liver and spleen.

To ascertain if this human strain of *B. violaceous* possessed an exotoxin, cultures were grown in flasks of broth. After incubation for 24 and 96 hours, the cultures were filtered through a Seitz filter at these respective periods. It was found that intraäbdominal injections of as much at 2 cc of this sterile filtrate into white mice produced no ill effects. When these animals were later injected with fatal doses of *B. violaceous*, they remained normal although control animals receiving the same dosage died overnight. Further tests for such protection were, therefore, made.

Animal Protection Experiments. Eight white mice were injected intraäbdominally with 0.5 cc of sterile broth Seitz filtrate obtained from a 24-hour culture. After 3 days, 1.5 cc of a similar filtrate from a broth culture grown for 96 hours, was likewise administered to these animals. Two days later all animals received intraabdominally 0.1 cc of a virulent *B. violaceous* broth culture. All animals survived and showed no evidence of illness during several weeks of observation.

Of 10 control mice, 5 received intraäbdominally 0.5 cc and 5 received 1.0 cc of the same culture as used for the above animals and all died overnight.

Two control mice were injected with plain sterile broth in similar amounts, route and time periods as those receiving the broth culture filtrate. The injection 48 hours later of 0.1 cc of the living culture produced death within 24 hours.

It is concluded from these observations that this strain of *B. violaceous* isolated from an unique clinical case, is pathogenic for various laboratory animals. When the inoculum permits of survival for several days, the lesions produced are similar in the gross and microscopic aspect to those of the human.

It is further shown that the injection of *B. violaceous* broth culture filtrates into white mice affords protection to doses of the living microörganism that kill control animals overnight.

Preliminary experiments with the serum of mice injected with sterile filtrates suggest that antitoxic factors are developed by which passive protection may be transferred to normal animals.

11852

A New Method for Regulated Vascular Obstruction.

PETER SALZBURG AND WILLIAM G. KUBICEK. (Introduced by M. B. Visscher.)

From the Department of Physiology, University of Minnesota, Minneapolis.

Restriction of blood flow in isolated vascular territories in living and unanesthetized animals has recently attracted attention. Some workers have fitted the blood vessels which are to be constricted into a type of rigid frame which reduces the diameter of the vessels.

Other investigators (Goldblatt,[1] Collins,[2] Rytand[3]) achieved the desired degree of ischemia by tightening loops of wire or thread around the vessel. Brotchner[4] and Blum, Schauer and Calef[5] employ a device which permits gradual mechanical closure of the vessel by means of 2 solid bars which are brought together by a screw to be actuated by a stiff wire from outside the animal.

The work of Mann, Herrick, Essex, and Baldes[6] correlated blood flow in vessels with a decrease in internal diameter brought about by local constriction. It was shown that a significant diminution of flow was achieved only after reduction of the internal diameter of arteries to a small percentage of the original.

The internal diameter of a vessel cannot very well be controlled quantitatively in the living animal because of the variability of the connective tissue reaction around the clamp among other reasons. Once a constricting loop or a rigid frame is applied to a vessel and the wound closed, the blood flow cannot be changed without further surgical intervention. Even then the percentage reduction of blood flow remains unknown until after the death of the animal, when model experiments can be performed on the flow of the blood in the constricted vessel.

The method which is described here attempts to regulate flow by employing the principle of the blood pressure cuff. This method should allow reversible and well defined obstruction of the vessels *in vivo,* when the pressure in the cuff can be controlled by a piece of rubber tubing which is brought out through the body wall.

Balloons of suitable dimensions have been obtained by the courtesy of Mr. H. E. Custer of the Dean Rubber Mfg. Co., 16th and Iron St., North Kansas City, Mo. If clamps of an irregular shape are needed it is relatively easy to make the balloons in the laboratory by dipping suitable molds into Latex* and drying. The finished clamp consists of a frame made of Lucite or Bakelite, one or two balloons which are fitted in to this frame and connecting glass and rubber tubing. The assembled instrument may be sterilized by boiling. The solid part has a U-shaped cross section and accommodates the vessel to be restricted in the lower part of the groove. The flat balloon is then put on top of and parallel to the vessel, and the groove is closed by a tightly fitting cover. The cover is held in place by a ligature. The balloon is fixed to the cover by doubling back the free end and tying it to the frame. Stretching of the thin latex balloon is avoided by slipping its open end on a glass tube and connecting the tube to the frame of the clamp. Very sensitive vessels which should not come in contact with the solid part of the clamp may be constricted between two inflatable balloons. In order to obtain a reduction of blood flow which can be repeated consistently and quantitatively it is essential to observe a few precautions during the operation and application of the clamp.

---

[1] Goldblatt, H., Lynch, Hanzal and Summerville, *J. Exp. Med.*, 1934, **59**, 347.

[2] Collins, D. A., *Am. J. Physiol.*, 1936, **116**, 616.

[3] Rytand, D. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 10.

[4] Brotchner, R. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 264.

[5] Blum, L., Schauer, G., and Calef, B., *Am. Heart J.*, 1938, **16**, 159.

[6] Mann, F. C., Herrick, J. F., Essex, H. E., and Baldes, E. J., *Surgery*, 1938, **4**, 249.

* Moulage No. 235, Vultex Chemical Co., 666 Main St., Cambridge, Mass.

The rubber balloon must be flaccid and without tension at atmospheric pressure. Both the artery and the balloon must fit tightly and snugly into the solid part. The balloon must fill the whole lumen of the U-groove without stretching of its walls. The artery must not be torn, constricted or kinked by the uninflated clamp. It is recommended to have available a series of clamps of varying cross-section in order to achieve the best results. Disregard of any of the above precautions may lead to results which are difficult to interpret.

Blood flow in the system composed of vessel and clamp is influenced by the following factors: pressure in the vessel, pressure in the constricting balloon, area of contact between cuff and vessel.

Of these factors the third has to be taken into consideration for the constriction of the clamp because the area of compression is determined by the length of the rigid part. If the area of contact is too small, pressures many times as high as the peak arterial pressures may fail to stop arterial flow completely. The cuff should compress the artery over a length of at least 8 mm in order to insure complete interruption of flow when the cuff pressure equals the peak arterial pressure.

The results reported below are taken from a femoral artery-femoral vein anastomosis in dogs. This arrangement eliminated peripheral resistance as a factor from our calibrations. For *in vivo* experiments the changes in peripheral resistance must be taken into account. Calibration over a range of pressure more extensive and continuous than reported can be undertaken in model circuits.

A Stolnikow-stromuhr had been interposed between artery and vein. The artery was constricted as described. The systolic and diastolic pressures were recorded optically by the "spoon" method (Sedgwick and Kubicek[7]). The manometer needle was inserted

---

[7] Kubicek, W. G., Sedgwick, F., and Visscher, M. B., *The Review of Scientific Instruments*, in press.

TABLE I.

Cuff pressure	Diastolic pressure	Systolic pressure	Flow (in % of unobstructed 120 cc/min)
0	120	170	100
14	120	170	100
32	130	180	66
66	125	175	50
66	135	175	50
88	135	175	33
104	140	175	20
122	135	175	18
132	135	175	10
143	135	175	8
156	135	175	5
165	130	175	3
174	135	170	2
188	135	175	1
209	130	175	0
150	125	170	2
140	130	170	3
128	125	175	3
112	125	170	7
94	130	175	9
81	130	180	10
109	95	140	5
85	95	145	9
30	105	150	40
8	110	150	55
74	115	160	20
136	120	160	6
173	115	155	0
160	115	155	15
103	115	155	10
5	110	150	70

longitudinally into the artery, cardiad to the constriction, with the bevel pointing towards the heart. The cuff pressure was read directly from a mercury manometer.

The reported figures indicate the gradations in flow which may be obtained with the above described device. It is believed that more satisfactory regulated and reversible restriction of blood flow can be achieved in this way than by other methods.

11853 P

Growth of the Mammary Glands in Hypophysectomized Mice.*

W. U. Gardner.

From the Department of Anatomy, Yale University School of Medicine.

Many investigations have indicated that the hypophysis is essential for mammary growth in animals receiving estrogen (see [1] and [2] for review). The above observations, however, have not been uniform in animals of different species or, in some instances, among animals of the same species observed in different laboratories. In hypophysectomized male mice the injection of large amounts of estrogens for prolonged periods[3] did not induce mammary growth. On the other hand mammary growth followed the injections of a lipoid extract of the pituitary gland and led to the assumption that the pituitary produced a mammogenic hormone.[4]

Recent experiments have demonstrated that estrogens will induce a local growth of the breast tissue of rabbits[5] and monkeys[6] if properly applied while adjacent mammary glands in the same animal are not affected. Similar experiments conducted with mice have given comparable results.[7] Mammary growth has also occurred in young male mice receiving injections of progesterone,[8] several androgens and desoxycorticosterone as well as estrogens.[9] The apparently contradictory observations listed above indicated the desirability of further studies on the estrogen-hypophyseal-mammary relationship.

One-hundred-one hybrid mice from 30 to 80 days old were hypo-

---

* These investigations have been supported by the Jane Coffin Childs Memorial Fund, the Anna Fuller Fund and the Fluid Research Funds of Yale University School of Medicine.

[1] Turner, C. W., *Mammary Glands*, Chapter XI, Sex and Internal Secretions, E. Allen, C. H. Danforth and E. A. Doisy, Editors, Williams & Wilkins, Baltimore; Nelson, W. O., *Physiol. Rev.*, 1936, **16**, 488.

[2] Fredrikson, H., *Acta Obst. et Gynec. Scand.*, **19**, Suppl. 1.

[3] Gomez, E. T., Turner, C. W., Gardner, W. U., and Hill, R. T., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 287.

[4] Lewis, A. A., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 435; *Mo. Agr. Exp. Sta. Res. Bul.* 282, 1938.

[5] Lyons, W. R., and Sako, Y., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 398.

[6] Speert, H., *Science*, 1940, **92**, 461.

[7] Gardner, W. U., *Yale J. Biol. and Med.*, in press.

[8] Gardner, W. U., and Hill, R. T., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 718.

[9] Van Heuverswyn, J., Folley, S. J., and Gardner, W. U., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 389.

physectomized using the parapharyngeal method described by Thomas.[10] Testosterone propionate, desoxycorticosterone acetate or progesterone† alone or in combination with estrogens or estrogen alone were administered for periods of 12 to 15 days starting from 1 to 89 days postoperatively. All hormones were dissolved in sesame oil and injected subcutaneously. The mice were fed Purina Fox Chow and water. The mammary glands were removed on the skins and prepared as "whole mounts". The completeness of hypophysectomy was checked by examination of serial sections of the sella turcica and surrounding tissues.

The 7 groups of mice in the present experiment are listed in Table I. Each of these groups was composed of smaller units of 3 to 6 animals. Each of the animals of these smaller groups received the same amount of hormone while the amounts varied among the groups. The range of the daily doses administered is indicated in the table.

All of the substances used induced mammary growth in hypophysectomized mice except the testosterone propionate. The relative number of mice showing mammary growth and the extent of mammary growth was greatest in those receiving desoxycorticosterone acetate + estradiol dipropionate, or progesterone + estradiol dipropionate. The glands were markedly enlarged, and from

TABLE I.
Summary of the Treatment and Mammary Response of Completely and Partially Hypophysectomized Mice Receiving Several Hormones.

Treatment hormones used	No. of mice	No. with complete hypophysectomy	No. with incomplete hypophysectomy	Treatment amt daily	No. of mammary responses: Complete hypophysectomy	No. of mammary responses: Incomplete hypophysectomy
TP*	16	11	5	.25 to 1.25 mg	0	1
DC	10	7	3	.25 mg	2	2
P	12	4	8	.125 to 0.25 mg	1	3
E	19	10	9	.05 μg to 1 μg	5	8
DC and E	18	14†	4	DC and E‡	12	3
P and E	16	12	4	P and E‡	11	4
TP and E	10	7	3	TP and E‡	0	0

*TP—Testosterone propionate; DC—Desoxycorticosterone acetate; P—Progesterone; E—Estradiol dipropionate.

†One mouse in this group had no mammary glands.

‡The amounts of the various hormones used in these groups varied as indicated above.

---

[10] Thomas, F., *Endocrinology*, 1938, **23**, 99.

† The testosterone propionate and progesterone were generously supplied by Dr. E. Schwenk of the Schering Corporation and the desoxycorticosterone acetate and estradiol dipropionate by Drs. Mautner and Oppenheimer of Ciba Pharmaceutical Products.

20 to 50 mitotic figures were counted in individual terminal buds of the proliferating ducts. The total size of the glands averaged less than those of intact animals similarly treated. The response of the glands of the incompletely operated animals was usually quite similar to that of the completely hypophysectomized except in a few instances. The very small amount of pituitary tissue remaining in some incompletely operated animals, no anterior lobe-tissue in some instances, did not greatly alter the quantitative response of the glands.

The amount of mammary growth occurring and the relative number of mice responding was smaller in the completely operated animals of the groups receiving desoxycorticosterone acetate, progesterone or estradiol dipropionate. The glands of these animals were only slightly enlarged and comparatively few dividing cells were present in the end buds of the ducts. The glands of the incompletely operated mice receiving desoxycorticosterone acetate, progesterone or estradiol dipropionate were all more extensively developed if anterior lobe tissue remained.

The complete lack of mammary growth in the mice receiving testosterone propionate + estradiol dipropionate cannot be explained at this time as both of these hormones alone induced a definite but slight mammary growth. The more extensive mammary proliferation of the mice receiving desoxycorticosterone acetate + estradiol dipropionate or progesterone + estradiol dipropionate is not considered to be only an additive effect.

Summary. Slight growth of mammary glands of some hypophysectomized male mice was induced within 15 days by the injection of desoxycorticosterone acetate, progesterone and estradiol dipropionate. A more extensive and more rapid proliferation of the mammary ducts of hypophysectomized mice occurred when desoxycorticosterone acetate or progesterone was injected with the estradiol dipropionate.

11854

Serological Relationship Between the Viruses of Japanese B and St. Louis Encephalitis.

MARGARET G. SMITH AND HAROLD R. REAMES. (Introduced by R. A. Moore.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Although many points of resemblance between the viruses of St. Louis and Japanese B encephalitis have been emphasized, the evidence supporting their serological relationship is conflicting.

In neutralization tests in mice, Kawamura[1] and his associates found no cross protection with antisera prepared in mice, rabbits and goats by immunization with the viruses of Japanese and St. Louis encephalitis, nor with sera of patients convalescent of the two diseases. Kasahara[2] and his colleagues found that the Japanese hyperimmune serum of rabbits neutralized both the Japanese virus and the St. Louis virus, but the latter only to a slight degree. On the other hand, the St. Louis immune serum neutralized only the St. Louis virus. With one of the strains of St. Louis virus used, this slight cross protection was apparent when the virus-serum mixtures were instilled intranasally but not when inoculated intracerebrally.

Kudo[3] and his collaborators observed that sera of rabbits immunized with the Japanese encephalitis virus neutralized not only the homologous virus, but to some extent the St. Louis virus. Their tables show that protection of mice against 1 to 100 minimal lethal intracerebral doses of the St. Louis virus was afforded by immune sera from each of 3 rabbits.

In protection tests in mice, Webster[4] found no cross neutralization of the Japanese and St. Louis viruses with the sera of individuals convalescent from either Japanese B encephalitis or St. Louis encephalitis. However, he cites an instance of cross neutralization with human sera noted by Kuttner.[5] Sera obtained by Kuttner from two Europeans who had contracted encephalitis in China protected against both the Japanese and the St. Louis viruses. Furthermore, Webster[4] obtained no cross protection against the Japanese virus with the serum of a monkey hyperimmunized with the St. Louis virus; but with serum from a monkey hyperimmunized with the Japanese virus, mouse protection was afforded not only against the Japanese virus but against at least one minimal lethal dose of the St. Louis virus. This cross protection with the Japanese serum is not emphasized by Webster, although it appears in the table of his results.

---

[1] Kawamura, R., Kodama, M., Ito, T., Yasaki, T., and Kobayakawa, Y., *Arch. Path.*, 1936, **22**, 510.

[2] Kasahara, S., Yamada, R., and Hamano, R., *Kitsato Arch. Exp. Med.*, 1937, **14**, 22.

[3] Kudo, M., Uraguchi, K., Matsuda, S., and Hashimoto, K., *J. Immunol.*, 1937, **32**, 129.

[4] Webster, L. T., *J. Exp. Med.*, 1938, **67**, 109.

[5] Kuttner, A., and T'ung, T., *J. Clin. Inv.*, 1936, **15**, 525.

In contrast to the results of others Perdrau,[6] in experiments published posthumously, demonstrated neutralization of the homologous and the heterologous virus to approximately the same extent with antisera prepared in rabbits by hyperimmunization with either the virus of Japanese encephalitis or the virus of St. Louis encephalitis. These results are not in accord with the other published data.*

In view of the conflicting evidence reported in the literature, it seems desirable to report our results obtained with the sera of mice and rabbits hyperimmunized with the viruses of St. Louis and Japanese encephalitis.

Materials and Methods. Virus. The Nakayama strain of Japanese encephalitis virus and the Hubbard strain of St. Louis encephalitis virus were employed in these experiments.

Serum. The serum was prepared in the following manner: Three groups of mice were immunized with the Japanese virus by repeated subcutaneous inoculations of .25 cc of dilutions of fresh infected mouse brain. Each group received 10 weekly inoculations. For the first inoculation a virus dilution of 10^{-5} was employed. The strength of the virus dilution used was increased with subsequent inoculations, so that a dilution of 10^{-2} was used for the final 4 inoculations. Each group of mice was bled one week after the last injection. The sera from the individual mice of each lot were pooled.

St. Louis virus immune serum was prepared in mice in a similar manner. Two groups of mice were immunized. One group received 11 inoculations; and the other, 14 inoculations. A virus dilution of 10^{-3} was used for the first few inoculations; and for later inoculations, a stronger dilution, 10^{-2}. The sera from the mice in each group were pooled.

Immune sera were prepared in rabbits by immunizing them with the Japanese and the St. Louis virus respectively by repeated intraperitoneal inoculations of active virus. Approximately 5 cc of a 10% dilution of virus in broth was used for each inoculation. Two animals immunized with the Japanese virus received 17 inoculations over a period of 10 months. Sera from these 2 rabbits were pooled. Another animal received 4 biweekly inoculations. Two rabbits immunized with the St. Louis virus received 20 inoculations over a period of 10 months. All rabbits were bled 2 to 3 weeks after the final inoculation.

---

[6] Perdrau, J. R., *J. Path. and Bact.*, 1940, **50**, 545.

* Webster has recently published the results of a study of the strains of virus used by Perdrau. He reached the conclusion that some of the material had been mislabeled and that Perdrau had been dealing only with strains of the St. Louis virus. Webster, L. T., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 499.

Neutralization Test. Brains of mice infected with the St. Louis or Japanese encephalitis virus were removed aseptically and stored in the frozen state for 1 or 2 days. At the time of the test, one brain was warmed at room temperature and made into a 10% emulsion with broth of pH 7.4. After light centrifugation the supernatant fluid was used to make serial tenfold dilutions. One part of each dilution of virus used was added to 2 parts of serum, and the virus-serum mixture was incubated for 2 hours at 37°C. Swiss mice under light ether anesthesia were inoculated intracerebrally with .03 cc of this mixture. Four mice were used for each dilution of virus.

Results. The results of the cross neutralization tests appear in the tables. It is apparent that no protection was afforded against the Japanese virus with either of the 2 lots of pooled St. Louis immune mouse sera, although good protection was afforded against the homologous virus (Table I). The same was true of the St. Louis immune rabbit sera (Table II). On the other hand, with each of the 3 lots of pooled Japanese immune mouse sera protection was

TABLE I.
Neutralization Tests Using St. Louis Immune Mouse Sera with the Japanese B Encephalitis Virus and with the St. Louis Encephalitis Virus.

	Dilution of Japanese virus added to serum			
Pooled mouse sera	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Normal No. 366	—	4, 6, 6, 8	(1), 6, 7, 8	6, 7, 7, S
St. Louis Imm. No. 342	—	(1), 6, 7, S	4, 5, 7, 8	(1), 6, 8, S
Normal No. 386	—	(1), 5, 5, 5	5, 6, 6, 7	6, 6, 6, 6
St. Louis Imm. No. 406	—	5, 6, 6, 6	5, 5, 6, 6	5, 7, 7, 7
	Dilution of St. Louis virus added to serum			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Normal No. 386	—	—	5, 5, 5, 5	5, S, S, S
St. Louis Imm. No. 342	—	S, S, S, S	S, S, S, S	S, S, S, S
St. Louis Imm. No. 406	S, S, S, S	13, S, S, S	S, S, S, S	S, S, S, S

Numerals indicate day of death of mouse; S = survival; () = accidental death.

afforded not only against the Japanese virus, but to a slight extent against the St. Louis virus (Table III). This protection against the heterologous virus, although slight against only one to 10 minimal lethal intracerebral doses, appears to be of significance since it occurred with all 3 sera. With the Japanese immune rabbit serum, the same slight neutralization of the heterologous virus was observed (Table IV). These results are in agreement with those obtained by Kudo and by Kasahara with immune rabbit sera. They are also in agreement with the findings recorded by Webster with immune

TABLE II.

Neutralization Tests Using St. Louis Immune Rabbit Sera with the Japanese B Encephalitis Virus and with the St. Louis Encephalitis Virus.

Rabbit sera	Dilution of Japanese virus added to serum				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Normal	—	—	5, 6, 7, 8	6, 8, S, S	—
St. Louis Imm. No. 391	—	—	(1), 6, 7, 7	7, S, S, S	—
Normal	—	—	5, 6, 6, 7	6, S, S, S	—
St. Louis Imm. No. 395	—	—	6, 7, S, S	S, S, S, S	—
	Dilution of St. Louis virus added to serum				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Normal	—	—	4, 4, 5, 5	4, 5, 5, 6	4, 5, 6, S
St. Louis Imm. No. 391	6, S, S, S	S, S, S, S	—	—	—
Normal	—	—	3, 4, 4, 5	4, 4, 5, 5	—
St. Louis Imm. No. 395	6, 6, 9, S	S, S, S, S	—	—	—

TABLE III.

Neutralization Tests Using Japanese Immune Mouse Sera with Japanese B Encephalitis Virus and with St. Louis Encephalitis Virus.

Pooled mouse sera	Dilution of St. Louis virus added to serum			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Normal No. 386	—	4, 5, 5, 5	(2), 5, 5, 8	5, 8, S, S
Japanese Imm. No. 385	—	5, 5, 5, 5	5, 5, 8, S	S, S, S, S
Normal No. 366	—	4, 4, 5, 5	5, 5, 7, 8	5, 6, S, S
Japanese Imm. No. 365	—	4, 5, 6, 10	5, 8, S, S	S, S, S, S
Normal No. 386	—	—	5, 5, 5, 5	5, S, S, S
Japanese Imm. No. 405	—	—	6, S, S, S	S, S, S, S
	Dilution of Japanese virus added to serum			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Normal No. 386	—	6, 6, 6, 6	6, 6, 6, 6	12, S, S, S
Japanese Imm. No. 385	—	8, 11, S, S	S, S, S, S	S, S, S, S
Normal No. 386	—	—	5, 6, 6, 7	6, 6, 6, 6
Japanese Imm. No. 405	6, 7, 8, 9	S, S, S, S	7, S, S, S	S, S, S, S

TABLE IV.
Neutralization Tests Using Japanese Immune Rabbit Sera with Japanese B Encephalitis Virus and with St. Louis Encephalitis Virus.

	Dilution of St. Louis virus added to serum				
Rabbit sera	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Normal serum	—	—	4, 4, 5, 5	4, 5, 5, 6	4, 5, 6, S
Japanese Imm. No. 399 (pooled)	—	—	5, 5, 5, 6	(1),5, 6, S	S, S, S, S
Normal serum No. 267	—	5, 5, 5, 5	5, 5, 5, 5	—	—
Japanese Imm. No. 270	—	5, 5, 5,10	5, S, S, S	—	—
	Dilution of Japanese virus added to serum				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Normal serum	—	—	5, 6, 7, 8	6, 8, S, S	—
Japanese Imm. No. 399 (pooled)	9, S, S, S	S, S, S, S	S, S, S, S	—	—

serum of the monkey, although the latter investigators apparently did not attach any significance to the findings.

An accumulation of evidence shows that in protection tests in mice, a definite though slight protection against the St. Louis virus by Japanese immune serum prepared in a number of animal species can be demonstrated. On the other hand, no protection against the Japanese virus is afforded by St. Louis immune serum.

This partial serological relationship between two viruses resembling each other in many other respects is analogous to the relationship which has been demonstrated among the 3 viruses—virus B, the virus of herpes, and that of pseudorabies.[7] The 3 latter possess many properties in common, and the antisera to these viruses give a slight cross neutralization of the viruses. However, with the antisera to the viruses of pseudorabies and of herpes, the partial cross neutralization is in one direction only. The immune antiherpes serum protects slightly against the virus of pseudorabies, while the antipseudorabies serum has no demonstrable effect against the herpes virus. It has been suggested that a generic relationship exists between these 3 viruses. It is possible that a similar group relationship exists between the 2 viruses producing the St. Louis and the Japanese type of encephalitis respectively.

A definite though slight protection against the virus of St. Louis encephalitis by serum of animals immunized with the Japanese virus has been demonstrated. On the other hand, no protection against the virus of Japanese encephalitis has been afforded by St. Louis immune serum.

---

[7] Sabin, Albert B., *Brit. J. Exp. Path.*, 1934, **15**, 372.

11855

Effect of Fluorine on Life Span of Rachitic Rats.*

SIDNEY B. FINN† AND MORTON KRAMER.‡ (Introduced by H. C. Hodge.)

From the School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

Although considerable study has been made of the toxic effects of fluorine upon the bones and teeth, little attention has been directed to its rôle in modifying phosphorus metabolism in certain of the deficiency diseases. The effect of fluorine upon the calcification of the long bones of rachitic rats has recently been studied roentgenographically by Morgareidge and Finn.[1] It was found that when rats were fed 300 p.p.m. of fluorine incorporated into a rachitogenic diet (Steenbock & Black 2965)[2] the rickets resulting was markedly less severe as evidenced by the narrower zone of hypertrophic metaphyseal cartilage and by the increased overall density of the tibial bone. With this apparent reduction in the severity of rickets it was interesting to speculate whether the increased density of the bone might be due to formation of a more radiopaque compound with fluorine in the bone or whether there was an actual lessening of the severity of the disease.

This experiment was undertaken to show the effect of fluorine upon the life span of rats fed a rachitogenic diet and to offer additional evidence on the specific effects of fluorine when added to such a diet. Twenty-six albino rats from our own inbred stock were divided equally at weaning age (21 days) into 2 groups. Litter mates and sexes were distributed equally. The first group was fed the rachitogenic diet (Steenbock & Black 2965) consisting of yellow corn 76%, wheat gluten 20%, $CaCo_3$ 3%, and NaCl 1%. The second group of rats was placed upon a similar ration plus 300 p.p.m. of dietary fluorine. This was incorporated by thoroughly mixing a saturated solution of NH_4F in 95% alcohol with the dry diet, spreading the wet mixture in thin layers on large sheets of wrapping paper and allowing the alcohol to evaporate. The amounts of inorganic phosphorus in each diet were determined by the colori-

---

* Supported by a grant from the Carnegie Corporation of N. Y.

† Now associated with the Maternity, Infancy and Child Hygiene Division, New York State Department of Health.

‡ Assistant Statistician, New York State Department of Health.

[1] Morgareidge, K., and Finn, S. B., *J. Nutrition,* 1940, **20**, 75.

[2] Steenbock, H., and Black, A., *J. Biol. Chem.,* 1925, **64**, 263.

metric method of Berenblum and Chain[3] and found to be equal, thus precluding any possible conversion of the organic phosphorus to the inorganic form by the action of the NH_4F and alcohol. Since the fluorine ration loses its efficacy on standing, this portion of the experimental diet was made up fresh every few weeks. The rats were given the diets and distilled water *ad lib.* until death occurred. Mottled enamel was observed to occur on the incisors of the rats on the fluorine supplemented diet. The age at death of each of the rats was recorded.

Findings. Table I gives the age at death (in days) of each of the rats and reveals that 10 of the rats fed the rachitogenic diet died before they reached the 68th day of life, whereas before that age no death occurred in the group of rats fed the rachitogenic diet supplemented with fluorine. By the end of the 75th day of life none of the rats fed the rachitogenic diet survived, while 9 of the rats fed the fluorine supplemented diet were still living.

The average age at death of the rats fed the rachitogenic diet was 65.23 days while for the rats fed the fluorine supplemented diet

TABLE I.
The Age at Death (in Days) of Each of the 13 Rats Fed the Rachitogenic Diet and of Each of the 13 Rats Fed the Rachitogenic Diet Supplemented with 300 p.p.m. Fluorine.

	Rachitogenic diet	Rachitogenic diet supplemented with fluorine
	Age at death (in days)	
	59	68
	60	73
	63	74
	63	75
	64	76
	64	76
	64	76
	64	78
	66	78
	67	80
	68	80
	71	82
	75	82
	—	—
Mean age at death (days)	65.23 ± 0.81	76.77 ± 0.73
Standard deviation (days)*	4.31 ± 0.57	3.90 ± 0.52

$$t = \frac{76.77 - 65.23}{1.61} = 7.16; \text{ degrees of freedom} = 24; \; P < 0.0001.$$

*The standard deviation is computed by the formula $s = \sqrt{\frac{\Sigma(\mathrm{x} - x)^2}{N - 1}}$ where x is the mean of the set of observations and N is the number of observations.

---

[3] Berenblum, I., and Chain, E., *Biochem. J.*, 1938, **32**, 295.

it was 76.77 days. When the difference between the means is tested by the t-Test,[4, 5, 6§] this difference of 11.5 days is highly significant, the probability of such a difference occurring by chance alone being less than 0.0001. The effect of fluorine on the life span of the rats thus appears to be real.

Summary. Fluorine, when added to a rachitogenic diet, appears to increase the life span of rats.

The authors wish to acknowledge the many suggestions of Drs. H. C. Hodge, K. Morgareidge, J. F. Volkart and D. B. Ast.

11856

Studies on Purified Tuberculin Prepared from Bacterial Bodies.

Sam C. Wong and George Ouyang.

From the Departments of Bacteriology and Immunology and Public Health, Peiping Union Medical College, Peiping, China.

Recent attempts[1, 2] to increase the yield of synthetic-medium tuberculin have been based primarily on increasing the growth-rate of the bacilli and on increasing the number of bacilli per unit-volume of medium. While admitting the importance of these factors it is generally agreed that further improvement along these lines is unlikely. A new approach made in this laboratory consists of increasing the production of tuberculin by the creation of an optimal condition in the medium favoring rapid cell-autolysis after maximal growth of organisms has been attained. Some of these findings have been reported.[3] Since the tuberculin thus obtained consists principally of bacillary proteins it seems of importance to compare the potency of purified tuberculin prepared from relatively pure bacillary proteins with that prepared from the soluble parts of the whole culture which is generally assumed to consist of metabolic products in addition to the bacillary proteins. On the other hand it

---

[4] Fischer, R. A., *Statistical Methods for Research Workers*, 5th Edition, pp. 120-125, 1934, Oliver & Boyd, London.

[5] Tippett, L. H. C., *The Methods of Statistics*, 2nd Edition, pp. 112-116, 1937, Williams & Norgate, Ltd., London.

[6] Yule, G. Y., and Kendall, M. G., *Introduction to the Theory of Statistics*, 11th Edition, pp. 438-443, 1937, Charles Griffin & Company, Ltd., London.

[§] A complete discussion of this test for determining the significance of the difference between two means is given in these references.

[1] White, W. C., *Am. Rev. Tuberc.*, 1934, **30**, 707.

[2] Wong, S. C., and Weinzirl, J., *Ibid.*, 1936, **33**, 577.

[3] Wong, S. C., *J. Bact.*, 1937, **33**, 451.

appears also of interest to determine the time required for complete extraction of the active cellular protein.

Methods. Preliminary trials have shown that autolysis of organisms was more rapid in dilute alkali than in the culture medium. In the present investigation studies were made exclusively on bacterial cells. Virulent *Mycobacterium tuberculosis,* H37, was grown for 4 weeks in liter Erlenmeyer flasks containing 300 ml of a malic-acid synthetic medium.[2] At the end of this period the fluid was decanted from each flask after which it was replenished with half of the original volume of sterile 0.05 N NaOH. For controls sterile distilled water was added to some of the flasks. All the flasks were reincubated at 37°C and the contents thoroughly shaken each day. The state of cellular disintegration was followed by examination of smears stained by the Ziehl-Neelsen technic every other day. When a majority of the bacilli had been found to have autolyzed each flask was tested for presence of contamination by culturing the fluid on blood-agar, pH 7.6, for 48 hours. The pH of 10 flasks was adjusted to 6.0 to 6.5 with 1-N HCl before heating at 100°C for 3 hours. The preparation will be designated as tuberculin A. Ten other flasks were heated without adjustment of pH and will be known as tuberculin B. The details of the preparation of purified tuberculin will be described elsewhere. Essentially the method is similar to that of Seibert's for PPD[4] with the exception that glycerol and dialysis in collodion sacs were omitted. Total nitrogen was determined by the micro-Kjeldahl method. The biological activities of both preparations were compared on human subjects with a PPD preparation kindly supplied to us by Dr. Florence Seibert. Only the first strength of the Mantoux test consisting of 0.00002 mg in 0.1 ml was compared. To rule out personal factors dilutions of all the tuberculins were done by one of us and issued to the other with different labels. The key was later supplied. Readings were made 48 hours after the injection by one of us but sometimes by a nurse. Injections were made on the dorsal surface of each forearm in approximately the same position. New tuberculin-syringes were used throughout this study.

Properties. Both preparations are light powders and very soluble in concentrations of 0.5% with the aid of few drops of dilute NaOH. The resulting solutions were pale yellow in color. The total nitrogen of tuberculin A was 13.27% and of tuberculin B, 12.96%. Incidentally it might be mentioned that the nitrogen-content of the former is the same as purified tuberculin prepared from the soluble parts of whole cultures. Intravenous injections of 15 mg contained in 1.5 ml into guinea pigs weighing 300-350 g produced no harmful effects. Prolonged immunization of rabbits, 150 mg per animal, showed that tuberculin B was non-antigenic while tuberculin A was weakly antigenic. In the latter case only complement-fixing bodies detectable in 1:5000 dilution of the antigen with serum diluted 1:5 was obtained.

---

[4] Seibert, F. B., *Am. Rev. Tuberc.*, 1934, **30**, 713.

Result. The yield of purified tuberculin in both cases was about 0.5 g. In contrast, the yield from the pooled supernate of 20 flasks was only 0.15 g. Autolysis began to appear on the 3d day of incubation and grossly this was manifested by the disappearance of granular forms. On the 14th day few intact cells could be found. On the other hand, stained specimens from control flasks in which distilled water alone was added showed comparatively little cellular disintegration. The heated tuberculin precipitated by trichloracetic acid in both preparations was practically salt-free, 3 to 4 washings being sufficient to render the precipitate free from chloride and sulfate ions. It was found that tuberculin B which had been heated at an alkaline pH was devoid of activity. This is to be expected since it is the classical procedure for hydrolyzing proteins. The result of the comparative biological activities of PPD and of tuberculin A which is prepared with pH adjusted to about 6 before heating is presented in Table I. From the table it is clear that tuberculin A is identical in activity to Seibert's PPD.

Comment. The above findings show 3 points of interest. First, evidence is at hand which seems to identify tuberculin with tuberculoprotein, since purified tuberculin prepared either from the bacillary protein or from the soluble parts of the whole culture presents similar biological, physical, chemical, and antigenic properties. Second, the method is only applicable if the pH of the crude tuberculin is adjusted

TABLE I.
Table Showing Biological Activity of Tuberculin Prepared from Acidified Lysate.

Subject	Readings in mm Seibert's PPD	Tuberculin A
H.T.C.	12 × 8	12 × 12
W.L.M.	13 × 10	14 × 11
F.H.	13 × 10	12 × 10
C.J.L.	15 × 15	15 × 12
C.T.Y.	0	0
L.C.L.	0	0
T.F.C.	17 × 15	13 × 12
L.L.L.	12 × 10	17 × 13
L.C.L.	17 × 15	15 × 12
F.Y.C.	12 × 11	13 × 12

to below 7 previous to heating. Conversely an alkaline pH at high temperature hydrolyses tuberculin and thereby destroys its biological activity. Third, the complete disintegration of the bacilli is a logical method in further increasing the yield of tuberculin, for the number of organisms present in most cultures grown in synthetic media is large.

Conclusion. From the results presented above it is justifiable to conclude that tuberculoprotein is suitable for the production of purified tuberculin and that a greater yield of tuberculin over a short period of time may be obtained if the organisms are treated with dilute alkali.

11857 P

Enhancing Action of Egg-Yolk on Virulence of Meningococcus for Mice.

K. H. PANG. (Introduced by S. H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

Nungester and his coworkers[1] found that gastric mucin of the hog exerts a great enhancing action on the virulence of meningococcus in experimental infection of mice. This observation has been widely confirmed and extended to a number of other microörganisms of low virulence. However, as Miller[2] and Nungester, *et al.*,[3] have pointed out that different lots of mucin varied considerably in their activities and that sterilization by excessive heat might decrease their action, difficulties have been met with in our hands to secure a potent product for experimental work. Recently, in our studies on rickettsiæ cultivated in the yolk-sac of the developing chick,[4] it was accidentally noticed that rickettsia in yolk was more virulent than the same organisms contained in other tissues. It was thought that possibly yolk has an enhancing action on virulence similar to that of mucin. In order to test this supposition, meningococcus has been chosen for study and the results are herewith communicated.

---

[1] Nungester, W. J., Wolf, A. A., and Jourdonais, L. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 120.

[2] Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1136.

[3] Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Inf. Dis.*, 1936, **59**, 11.

[4] Pang, K. H., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 76.

Materials and Method of Study. Two freshly isolated strains of meningococcus were employed for this study. Both were of low virulence when suspended in saline or Tyrode's solution. Organisms were grown on blood-agar plate for 6 to 16 hours and made up in Tyrode's solution to 500 million organisms per ml as compared with "Wellcome" opacity-tube for the standardization of bacterial vaccines. Decimal dilutions were then made also in Tyrode's solution to obtain decreasing numbers of organisms and were then mixed with 9 volumes of diluted egg-yolk immediately before inoculation.

Fresh egg-yolk was obtained aseptically and emulsified into uniform consistency by shaking vigorously with sterile glass rods. This emulsion was very sticky and its relative viscosity was found to be over 60 as determined with an Oswald's viscosimeter. Emulsions of varying degrees of viscosity were prepared by the addition of different amounts of sterile Tyrode's solution as listed in Table I.

TABLE I.
Relative Viscosities of Different Dilutions of Egg-yolk.

Relative viscosity	60	52.5	43	34	25.5	19	14.5	11	8.5	7	6.4	5
Egg-yolk in ml	5	4.6	4.4	4.2	4.0	3.8	3.6	3.4	3.2	3.0	2.8	2.5
Tyrode's in ml	0	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.5

Yolk-emulsions possessing relative viscosity between 11 to 19 have been found to be most satisfactory. They were effective in enhancing the virulence of the organisms and, at the same time, sufficiently dilute to be used for inoculation employing a 27-gauge needle.

Results. Yolk-emulsion alone in 2 ml amounts of any dilutions produced no harmful result after intraäbdominal injection into white mice. On the other hand, animals receiving as few as 10 meningococci suspended in the same amount of yolk-emulsions of proper viscosity showed marked prostration, ruffling of hair, discharge from eyes, and occasional convulsion and tremor. Death, preceded by coma, usually took place in 16-48 hours. Severe bacteremia could be detected by culturing a drop of blood from the tail vein either just before or soon after death of the animals. However, when the amount of egg-yolk emulsion, irrespective of its viscosity, was decreased this enhancing action on the virulence of the organisms was also greatly reduced. If 1 ml was used instead of 2 ml to suspend the organisms, at least 1000 times more organisms were required to kill mice. As the amount of yolk-emulsion was further decreased below 0.5 ml it became practically inert.

Comment. Emulsions of egg-yolk of certain viscosity have been found to exert an enhancing action on the virulence of 2 freshly isolated strains of meningococcus for white mice when the proper amount of yolk-emulsion was employed and the intraäbdominal route was used for inoculation. As little as 10 organisms were able to kill white mice in 16-48 hours. In contrast to the varying quality of gastric mucin, egg-yolk is easily obtainable and the material has been found to be uniform in pH value and in viscosity as long as the eggs were fresh. If aseptic care is taken in handling the material, no further sterilization is required. Emulsions of the desired viscosity may be prepared according to the data given.

11858

Immunological Studies on Proteins of *Corynebacterium diphtheriæ.*

SAM C. WONG.

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

Previous studies[1] have shown that type-specific protein derived from the Park-Williams 8 strain of *C. diphtheriæ* may be converted into a group-specific protein by heating at 56°C for 30 minutes. This finding suggests that diphtheric proteins in general may not be a single antigen. However, direct chemical methods, such as fractional precipitations with ammonium sulfate, have, thus far, not revealed any antigenic differences. Likewise the application of the sensitive complement-fixation reaction has proven impractical since rabbit sera immunized with whole organisms are markedly anticomplementary and diphtheric proteins alone failed to produce potent antiserum.[1] If it were possible to enhance the antigenic quality of the type-specific protein in rabbits by adsorbing the protein to charcoal[2] the usual serological methods could then be applied. The results of such a study are described below.

Method. Proteins were prepared[1] from representative strains of the serological types D41 and D43 and of group x.[3] All were serologically active, giving precipitin-titers of 1:100,000 with the specific serum only. The method of preparing the charcoal for absorption was as follows: 0.2 g of animal charcoal was suspended and washed with 3 changes of sterile distilled water followed by 3 of sterile physiological saline. It was then suspended in about 30 ml of saline and left at 56°C for 2 hours. After removing the supernate by centrifugation, 15 cc of a 1% solution of the type-specific protein were added and thoroughly mixed. It was placed in a waterbath at 37°C for 2 hours with frequent agitation. Then 25 ml of sterile saline were added to the mixture and it was refrigerated. The subcutaneous route of injection was employed after preliminary trials had shown it to be the most effective. Only rabbits whose sera did not contain natural antibodies and were not anticomplementary were used. Two animals were employed for each protein. Injections were made on 2 successive days followed by 5 days of rest and a total of 8 injections; totalling 16 ml, was given to each animal. Animals were bled 7 days after the last injection.

---

[1] Wong, S. C., and T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 824.

[2] Seibert, F. B., *J. Immun.*, 1935, **28**, 425.

[3] Sia, R. H. P., and Huang, C. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 348.

Result. All the immune sera gave specific agglutinative reactions. The precipitin titers varied from 1:10,000 to 1:50,000 when undiluted serum was overlayered with varying dilutions of the antigen and all but one gave reaction only with the homologous protein. Type D41 immune serum, however, gave marked cross-precipitation with the group x protein. The reverse of this, namely, the reaction between group x immune serum and type D41 protein did not occur. On the other hand, when a more sensitive method such as complement-fixation was employed the sera were found to be less specific, marked cross-reactions among the various proteins and sera being found. The results showing the antigenic complexity of the diphtheric proteins are presented in Table I. An examination of the last column of the table shows that the diphtheric proteins contain at least 2 antigens, one specific, and a common one shared apparently by all types. The specific antigen is best shown with type D41 protein which gave no reaction with group x serum and only a weak reaction with type D43 serum, while the common antigen is best shown by the group x protein which reacts to the same degree with the homologous as well as heterologous immune sera. An exception to this is found in the immune serum of type D41 which reacted strongly with the type-specific protein of type D43. This may be due to the fact that rabbits were superimmunized and therefore this serum was unsuitable for the demonstration of the type-specificity. In general, however, the complement-fixation titers of all sera were higher with homologous protein than with heterologous ones. It is of interest to note that serologically inactive proteins of

TABLE I.
Antigenic Relationship of Diphtheric Proteins.

Immune serum	Protein	Precipitin-titer	Complement-fixation titer
Type D41	T41	1:10,000	1: 100,000
	G x	1:10,000	1: 100,000
	T43	Negative*	1: 50,000
Group x	T41	Negative*	Negative*
	G x	1:10,000	1: 500,000
	T43	Negative*	1: 10,000
Type D43	T41	Negative*	1: 10,000
	G x	Negative*	1: 50,000
	T43	1:50,000	1:1,000,000

*Negative in 1:1000 dilution of the antigen.

C. diphtheriæ[4] (1:1000) can not be rendered antigenic by adsorption to charcoal. The polysaccharides of *C. diphtheriæ* did not give precipitin-reactions with any of the sera but did have a complement-fixing titer of 1:10,000.

Comment. Taking the above findings in addition to those reported elsewhere[1] we may be justified in arriving at the following conclusions regarding the antigenic structure of diphtheric protein. The protein is composed of at least a heat-labile type-specific antigen and a heat-stable common antigen shared by all diphtheric organisms. The specific antigen being present in larger quantity masks the reaction of the other in the precipitative tests. The common antigen, however, may be manifested either in the serum of superimmunized animals by complement-fixation tests or by inactivating the protein with heat. The specific antigen is also lost when a diphtheric organism loses its virulence as was found in the case of the protein prepared from the avirulent organisms belonging to group x. Incidentally the method of adsorbing type-specific protein to charcoal may be useful in the production of type-specific serum since even undiluted serum did not cross-agglutinate.

Conclusion. Diphtheric protein is a complex antigen consisting of at least a type-specific antigen and a group-antigen.

---

[4] Unpublished results.

11859

Choleretic Action and Excretion of Cinchophen in Rabbit Bile.

A. L. Berman and J. H. Ivy. (Introduced by A. C. Ivy.)
From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Since the report of Brugsch and Horsters[1] many investigators have definitely proved that cinchophen, or 2-phenylquinoline-4-carboxylic acid, increases the volume output of bile in dogs and man. The choleretic effect of cinchophen in the rabbit has been disputed in the literature. Stransky[2] reported no increase in volume output in anesthetized and unanesthetized rabbits in doses of 0.05-0.2 g. Franke[3] reported that small doses of atophan, up to 0.1 g, had no effect on bile flow in anesthetized rabbits, whereas larger doses, 0.15 to 0.6 g, increased the bile flow. Matsuoko[4] noted a choleretic effect of atophan in rabbits.

Our work was undertaken to determine the effect of cinchophen on bile flow in anesthetized rabbits, and to correlate the effect with the amount of cinchophen excreted in the bile.

Methods. Rabbits weighing from 1.0 to 2.5 kilos were anesthetized with nembutal (sodium pentobarbital). The abdomen was opened and the common duct cannulated near its entrance into the duodenum. The cystic duct was then ligated securely. The bile was allowed to flow for about one hour until the volume had reached a constant basal level. Cinchophen (Calco), as the sodium salt, was injected into the femoral vein in doses of 50, 25 or 10 mg per kilo of body weight. The bile was then collected at half-hour intervals for 2 hours, and the volumes noted and recorded. At the end of the experiment the bile secreted after the injection of cinchophen was pooled, so that the amount of cinchophen secreted could be determined. The method devised by Bradley[5] was used for the determination of cinchophen in bile.

Results. Fifty mg of cinchophen were injected slowly intravenously into 11 rabbits. The injection of cinchophen did not cause an increase in bile volume output. The average control output of bile for the 11 rabbits was 2.6 cc per half-hour, while the average output for the first half-hour after the injection of cinchophen was 2.4 cc. During the remainder of the test period, the output usually showed a gradual decrease, or showed normal fluctuations. A normal variation of ±17% in volume output was noted in 5 control rabbits which received no cinchophen. The injection of 50 mg per kilo of sodium dehydrocholate 2 hours after the cinchophen provoked a brisk choleresis.

---

[1] Brugsch, T., and Horsters, H., *Z. Ges. Exp. Med.*, 1923, **38**, 367.

[2] Stransky, E., *Biochem. Z.*, 1925, **155**, 256.

[3] Franke, K., *Arch. Exp. Path. u. Pharm.*, 1930, **151**, 219.

[4] Matsuoko, Y., *Japan. J. Gastr.*, 1936, **8**, 145.

[5] Bradley, W. B., Ivy, A. C., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 143.

It was thought that possibly a dose of 50 mg per kilo was too large for rabbits. Therefore, in subsequent experiments 25 and 10 mg of cinchophen per kilo were injected. In 5 rabbits, 25 mg per kilo gave no choleretic response. Similar results were obtained with 10 mg of cinchophen per kilo in 3 rabbits.

It was quite obvious that, under the conditions of our experiments, cinchophen in large or small doses manifested no choleretic property in rabbits. Bradley[5] in his studies on anesthetized and unanesthetized biliary fistula dogs found that the increase in bile volume occurring with the administration of 50 mg of cinchophen per kilo was associated with the recovery in the bile of approximately 20 to 78% of the administered cinchophen. Thinking that this fact, confirmed by us during this study, may be concerned in the mechanism of cinchophen choleresis, the bile obtained during the experiment was analyzed for cinchophen content.

The total amount of cinchophen excreted by 11 anesthetized rabbits when 50 mg per kilo was administered was 32.7 mg or an average of approximately 3 mg per rabbit during the collection period of 2 hours. In anesthetized dogs, Bradley[5] recovered 51 mg of cinchophen per dog during the same period of time. When the smaller doses were administered correspondingly smaller quantities of cinchophen were recovered in the bile. No cinchophen was found in the control bile, nor in the bile obtained after the injection of sodium dehydrocholate (Decholin-Sodium) which is an excellent choleretic in rabbits according to our observations on 12 rabbits. When small amounts of cinchophen were added to bile approximately 100% recovery was obtained. It is remotely possible that the liver of the rabbit is more sensitive to sodium pentobarbital anesthesia than the liver of the dog, even though the pentobarbitalized rabbit's liver secreted briskly in response to sodium dehydrocholate. To test this possibility 50 mg of sodium cinchophen per kilo was given slowly intravenously to 3 unanesthetized (chronic) biliary fistula rabbits. A choleresis did not occur.

Discussion. From our results it can readily be concluded that different doses of cinchophen had no appreciable effect on the volume output of bile in rabbits. Our next problem was to find the reason for this non-choleretic action of cinchophen, especially since it was at variance with the results obtained in dogs. It seemed to us that cinchophen in the doses employed did not acutely damage the liver, because sodium dehydrocholate injected after the administration of cinchophen always caused a marked increase in volume output. A study of the literature revealed that, generally, rabbits are less susceptible to cinchophen poisoning than other mammals. Myers and Goodman[6] reported that 300 mg of cinchophen administered to rabbits for 45 days caused only minor cellular changes in the liver. Hanzlik and Lehman[7] found no change in the rose bengal liver function test after feeding 18 rabbits from 0.3 to 1.6 g of cinchophen per kilo over a period of 7 to 89 days. They also found that cinchophen did not increase the sensitivity of the liver to chloroform or phosphorus poisoning. Schwartz and Simonds[8] were unable to obtain peptic ulcers in rabbits fed 220 to 550 mg of cinchophen per kilo per day for 76 to 99 days.

From our results on the recovery of cinchophen in the bile, it appears that the absence of choleresis in rabbits may be associated with the observation that only very small amounts of cinchophen are excreted in the bile. We have found, in previous investigations[9, 10] that the choleresis obtained from the administration of various bile salt preparations was associated with the presence of appreciable amounts of the bile acid in the excreted bile. In the rabbit the liver is not concerned with the excretion of cinchophen as in the dog. Since cinchophen is not excreted in significant amounts in the bile of the rabbit, it would exert no osmotic effect on the bile and extra water would not be excreted in the bile. The failure of the rabbit's liver to excrete cinchophen is the only available explanation of the fact that the drug fails to cause choleresis in the rabbit.

Summary and Conclusions. The chloretic action of various doses of cinchophen was studied in a relatively large number of anesthetized rabbits. Cinchophen in doses ranging from 10 to 50 mg per kilo had no appreciable effect on the volume output of bile. It was also found that, on the average, only 3 mg of cinchophen per rabbit was recovered in the bile over a period of 2 hours. Since it was found that the absence of cinchophen choleresis in the rabbit was not due to an acute intoxication of the liver, because after cinchophen sodium dehydrocholate caused a brisk choleresis, it is tentatively concluded that cinchophen does not cause choleresis in the rabbit because it is not excreted in the bile.

---

[6] Myers, H. B., and Goodman, L., *Arch. Int. Med.*, 1932, **49**, 946.

[7] Hanzlik, P. J., and Lehman, A. J., *Arch. Int. Med.*, 1933, **52**, 471.

[8] Schwartz, S. O., and Simonds, J. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1133.

[9] Berman, A. L., Snapp, E., Ivy, A. C., Atkinson, A. J., and Hough, V. H., *Am. J. Dig. Dis.*, 1940, **7**, 333.

[10] Berman, A. L., Snapp, E., Ivy, A. C., Hough, V. H., and Atkinson, A. J., *Am. J. Physiol.*, in press.

11860

Radioactive Ion Distribution in Protoplasmic Granules.

LORIN J. MULLINS. (Introduced by S. C. Brooks.)
From the Department of Zoology, University of California, Berkeley.

The *Nitella* cell has an extensively granulated protoplasm, measurements on frozen sections giving up to 15% of the total volume of the protoplasm as being made up of nucleii, plastids, mitochondria and non-ergastic bodies. It seems important to inquire whether these granules play a significant rôle in ion transfers, and indeed, whether these bodies might not be the main seat of ion accumulation.

To determine to what extent the granuloplasm took up ions, single internodal coenocytes 5-10 cm in length of *Nitella coronata* were prepared by destroying alternate cells in the chain. Care was taken to preserve a short extension of the cellulose wall (about 5 mm long) from the adjacent cell. Surgical thread could then be tied around this cellulose projection and the cell left for 24 hours in pond water in the cold room (15°C) under conditions of intermittent light which simulated sunlight. After this treatment the cell was immersed in radioactive solutions for the desired time and then placed in a centrifuge tube, the thread tied about a cork in the tube and its length adjusted so that the cell was held clear of the sides and bottom of the tube. Centrifugal force sufficient to displace the granules in the cell was applied without any other observable damage. Control centrifuged cells kept under the previous conditions would show a gradual redistribution, reaching substantial completion in about 60 hours, of the stratified granules. These cells lived as long as untreated cells. After stratification had been obtained the experimental cells were bisected into centripetal and centrifugal halves, or fractions, and the activity of each half of the protoplasm measured under a Geiger-Muller counting tube. The only apparent error due to mixing was in the sap, which almost certainly became mixed during the sampling.

Preliminary studies showed that relatively great centrifugal forces (4,000 rpm at 15 cm) were necessary to displace the plastids in the cortical plasmagel whereas to displace the granules in the mobile plasmasol relatively small centrifugal forces (1,000 rpm at 15 cm) were required. As can be seen from the tables these cortical plastids (mostly chloroplastids) apparently were inactive as ion accumulation centers, and only served to dilute the granule fraction in general. However, when centrifugation was sufficient the cytoplasm became absolutely clear showing that all granules were sedimented.

The data obtained were quite reproducible and the maximum error did not exceed ± 5%. Tables I and II show the change K^* or $HP^*O_4 =$ ion concentrations in the granuloplasm after various periods of immersion of the cell in 0.01M K^*Cl or $Na_2HP^*O_4$ in pond water at pH 8.2 temperature 15 ± .01°C. Control cells, those which had not been centrifuged, were immersed in radioactive salts and then cut in half and each half measured for radioactivity. This showed that these halves each had the same ion radioactive concentration.

The times of sampling were chosen to correspond with various phases of the ion penetration process, namely: .25 hr corresponding to a maximum of induced accumulation (*cf.* Brooks[1]); .5 hr corresponding to a minimum of ion concentration, and 4-8 hr cor-

TABLE I.

Granule Accumulation Ratios for Various Times of Immersion of Cells in Radioactive Solutions.

Ratio: $\frac{K^* \text{ Ion Concentration in Granular Protoplasm}}{K^* \text{ Ion Concentration in Hyaline Protoplasm}}$		Time of immersion, hr
Low centrifugal force	High centrifugal force	
0.30	0.20	0.25
0.64	0.55	0.50
1.10	0.60	1.00
2.20	0.75	4.00

TABLE II.

Ratio: $\frac{HP^*O_4^{=} \text{ Concentration in Granular Protoplasm}}{HP^*O_4^{=} \text{ Concentration in Hyaline Protoplasm}}$ (Low centrifugal force)	Time of immersion hr
0.80	0.25
1.48	0.50
2.80	1.00
4.44	2.00
10.55	8.00

---

[1] Brooks, S. C., *J. Cell. Comp. Physiol.*, 1939, **14**, 4.

responding to true primary accumulation of ions by the cell. It appears that as the accumulation of ions progresses, the concentration of these ions takes place in the granules of the cell. An objection to this technic may be that the whole protoplasm is centrifuged to the bottom of the cell under the conditions of the experiment. This, however, does not seem likely in view of the relatively strong turgor of the cells and the low centrifugal forces applied. A general discussion of ion permeability phenomena will be found in Mullins[2] so that only a brief explanation of the data will be given here.

Since a large concentration of ions distributed throughout the hyaline protoplasm might alter markedly the colloidal nature of the protoplasm by causing excessive solation of the colloid, it seems reasonable that there must be some stations in the cell where ions are accumulated and rendered ineffective. Apparently the vacuole of the *Nitella* cell functions in this manner, and the data here given would indicate that the granules also supplement the vacuole in this process.

11861

A Micro-Photoelectric Photometer.

A. A. WEECH.

From the Department of Pediatrics, College of Physicians and Surgeons, Columbia University, New York.

Photoelectric analytical procedures depend for accuracy upon a depth of color in solution sufficient to produce satisfactory deflection of a galvanometer or other recording device. Ideally the volumes of reagents and sample are adjusted to yield 20-80% absorption of the incident light. Since the necessary intensity of color varies with the length of the path of the light beam in the solution, the dimensions of the absorption cell are limiting factors in adapting procedures to microanalysis. Present instruments on the market do not permit an absorption path longer than 20 mm when the volume of solution is limited to 1 cc.

An instrument in use at the Babies Hospital during the past 2½ years demonstrates the feasibility of constructing apparatus in which the light beam traverses a path of 50 mm through a tube-cell of narrow bore and only 1 cc capacity. These specifications have made it possible to apply the photoelectric principle to measuring the low intensities of color in the cholesterol micromethod of Schoenheimer and Sperry[1] and have allowed the development of a technic for determining bilirubin in serum when the sample is only 0.1 cc. The sensitivity is great enough to make obligatory the use of color filters of narrow spectral transparency. Since no basically new features are involved in the electrical or optical systems, the instrument is adequately described by the details in Fig. 1. The light source is a 15-candle-power automobile bulb. The ratchets, not shown in the diagram, for centering the position of the lamp must be capable of fine lateral and horizontal adjustments in order that the beam shall traverse the absorption cell and fall on the dispersing lens, M. Energy for

---

[2] Mullins, L. J., *Ionic Equilibria in Protoplasm*, in press, 1940.

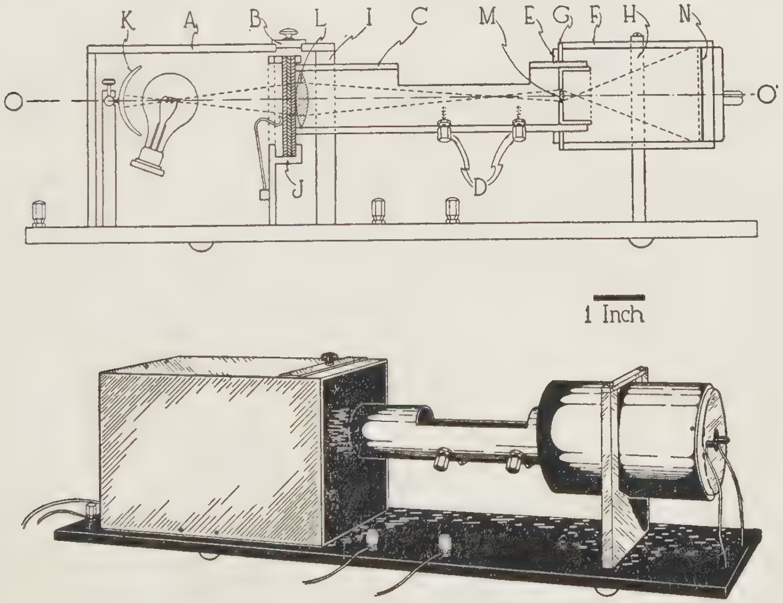


FIG. 1.

Diagrammatic section through the photoelectric photometer and three dimensional sketch of the instrument. The labeled parts of the diagram refer to: A, housing for the light compartment; B, lid over window giving access to the color filters; C, brass tube forming the cell compartment; D, bolts for supporting the absorption cell; E, lock-nut for fixing position of the cell compartment; F, compartment for the photoelement; G, end plate of the previous compartment; H, brass support; I, Bakelite support; J, compartment for the color filters; K, spherical mirror (diameter $1\frac{9}{16}''$, radius of curvature $1\frac{1}{4}''$); L, converging lens (diameter 30 mm, focal length 37 mm); M, dispersing lens (diameter 6 mm, focal length 6.4 mm); N, photoelement (Lange, type S-50 or Weston photronic, type 2); OO′, optical center of the instrument.

---

[1] Schoenheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, **106**, 745; Sperry, W. M., *Am. J. Clin. Path.*, 1938, **8**, 91.

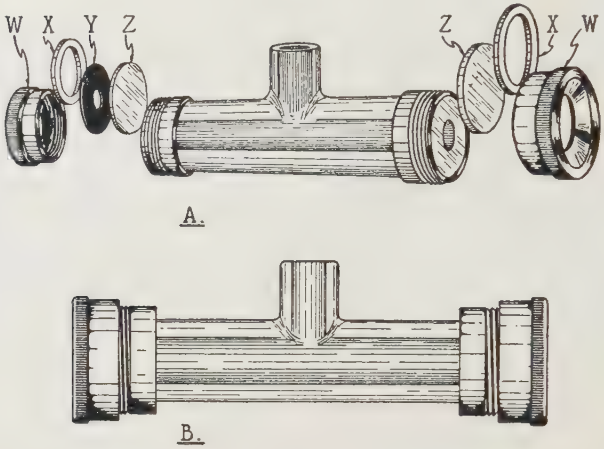


Fig. 2.

The absorption cell of 1 cc capacity; A indicates the constituent parts of the cell and B, the assembled cell. In addition to the main body of the cell, the parts include: two metal caps, W; two rubber washers, X; a single metal diaphragm, Y; two glass windows, Z.

the lamp is furnished by a 6-volt storage battery in series with a sliding-contact rheostat of 0.83 ohm resistance. The photocurrent is measured with a Lange multiflex galvanometer (maximum sensitivity, 4 x 10^{-9} amperes per mm of scale; maximum internal resistance, 1000 ohms).

The absorption cell, Fig. 2, can be purchased as part of the accessory equipment of the Zeiss Pulfrich photometer. For noncorrosive solutions we use a cell of similar dimensions constructed of German silver and encased in an insulating barrel of plastic. The fidelity of readings is somewhat greater with the metal than with the glass cell, apparently because equilibration of temperature is attained more rapidly. The analytical methods examined so far have utilized organic solvents for the development of color; with such solutions there has been no difficulty in rinsing and filling the narrow-bore cell. In operation the cell is filled with a blank solution of the reagents used in the method, placed in the instrument and rotated to a fixed and reproducible position. Galvanometer sensitivity is adjusted to yield full deflection of 100 scale divisions. Because the cell acts as a condensing lens, its removal brings about a fall in the galvanometer deflection. This "air-setting" is noted and maintained throughout subsequent readings by adjustment of the galvanometer rheostat. The percentage light transmission of the unknown solutions can then be read directly as units of deflection on the scale.

For the purpose of this report the reduplicability of measures of color intensity was determined for the German silver cell by making a long series of readings over a number of days on two standard intensities of color. The colored solutions were composed of pure carotene dissolved in chloroform and diluted with alcohol; the readings were made with a blue filter. With the more dilute solution light transmission was about 70%; concentrations calculated at this level showed a standard error of ± 0.65%. With the more concentrated solution light transmission was approximately 25%; concentrations computed from these data disclosed a standard error of ±0.24%.

The author expresses his indebtedness to Mr. Kern F. Larkin, who solved most of the constructional problems and to Mr. Fred Rosebury, who assisted in this work.

11862 P

Differentiation of Influenza A and Influenza B by the Complement-Fixation Reaction.*

THOMAS FRANCIS, JR.

From the Department of Bacteriology, New York University College of Medicine.

A recent report[1] has described the isolation of a new serological type (B) of virus from epidemic influenza. By means of the neutralization test with sera from convalescent animals and human individuals, it was shown that this virus was readily differentiated from virus of the type (A) previously identified. In the process of adaptation of the new virus to mice, attempts were made to ascertain whether serological differentiation could also be obtained with the complement-fixation reaction. While the earlier results were indefinite, these efforts have been renewed since the virus has increased in virulence for mice.

Antigens were prepared from lungs of mice infected with the PR8 strain of the virus of Influenza A[2] and also from mice infected with the Lee strain of Influenza B virus. Two percent suspensions of the infected lungs in physiological salt solution, after clarification by centrifugation, were used in the tests. Two units of complement were employed. Serial two-fold dilutions of serum were made and 0.1 cc of each dilution was mixed with 0.1 cc volumes of complement, antigen and saline. After incubation for 60 minutes at 37°C., 0.2 cc of sensitized cells were added. The tubes were again incubated in the waterbath for 45 minutes when readings were made. A complete set of controls was included in each test.

---

* This study was conducted under a grant from the International Health Division of the Rockefeller Foundation.

[1] Francis, T., Jr., *Science*, 1940, **92**, 405.

[2] Horsfall, F. L., Jr., Lennette, E. H., Rickard, E. R., Andrewes, C. H., Smith, W., and Stuart-Harris, C. H., *Lancet*, 1940, **2**, 413.

Acute and convalescent sera obtained from patients infected with Type A virus in 1938-39 and from others shown to have been infected in 1940 with Type B virus were tested for their capacity to fix complement in the presence of each antigen. Representative results are presented in Table I, where the titer of neutralizing antibodies in each serum against 100 M.L.D. of the 2 types of virus is also recorded.

It is seen that the convalescent sera of patients who had suffered from epidemic influenza in 1938-39 showed an increase in complement-fixing antibodies to the PR8 strain of Influenza A virus but not to the Lee strain of Influenza B virus. On the other hand, the convalescent sera from 4 of the patients suffering from epidemic

TABLE I.
Complement-Fixation with Sera of Patients and Influenza Virus, Types A and B.

			Type A (PR8) antigen, Serum dilution 1:						Type B (Lee) antigen, Serum dilution 1:						Neutralization titer	
Type of Infection	Pt.	Serum	2	4	8	16	32	C	2	4	8	16	32	C	Type A	Type B
Influenza A 1938-1939	V	A*	4	2	0	0	0	0	4	1	0			0	6	0
		C	4	4	4	4	1	0	4	1	0			0	240+	0
	J	A	1	0	0	0		0	3	0	0			0	4	6
		C	4	3	1	0		0	1	0	0			0	140	6
	A	A	4	0	0	0	0	0	3	0	0			0	30	0
		C	4	4	4	2	0	0	1	0	0			0	240	6
	L	A	0	0	0			0	0	0	0			0	120	6
		C	4	3	0			0	0	0	0			0	480	6
	Ac	A	0	0	0			0	0	0	0			0	30	8
		C	4	2	0			0	0	0	0			0	120	15
Influenza B 1940	M	A	0	0	0			0	0	0	0	0	0	0	17	0
		C	0	0	0			0	4	4	3	2	0	0	17	60
	T	A	0	0	0			0	0	0	0	0	0	0	10	12
		C	0	0	0			0	4	4	4	3	0	0	16	240
	C	A	2	1	0			0	3	1	0	0	0	0	240+	6
		C	3	1	0			0	4	4	4	4	4	0	240+	120
	L	A	4	4	1	0		0	2	1	0	0	0	0	240+	0
		C	4	4	1	0		0	4	4	4	4	1	0	240+	200
	S	A	0	0	0			0	0	0	0			0	240+	0
		C	2	0	0			0	0	0	0			0	240+	140

0 = no fixation of complement.
1, 2, 3, 4 = increasing degrees of fixation.
*A = acute, C = convalescent.

influenza in 1940 showed a sharp rise in antibodies to the Type B antigen but not to the antigen of Type A. In the case of patient Sq., no rise in complement-fixing antibodies was detected.

These observations, while limited in number, serve to emphasize the antigenic differences between the 2 viruses and to indicate that the complement-fixation reaction can be employed for the differential diagnosis of epidemic influenza due to virus of Types A and B.

11863

The Diurnal Levels of Blood Leukocytes in the Normal Rabbit.

ALBERT E. CASEY.

From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University.

Extensive studies on the blood of normal rabbits,* which were made for another purpose,[1, 2] seemed to offer an unique opportunity to determine whether a definite diurnal cycle in the leukocyte level actually exists in this species. The animal material used had been acclimated to the laboratory for months or years before the counts were made. The exact age of the animals, as well as their breed and physical condition, was known in most instances. Counts were not made oftener than 3 times a week, and usually only once a week. The exact time of day at which the blood was taken had been recorded for each determination. Finally, the material was sufficiently extensive to make statistical analysis possible.

Materials and Methods. Although blood counts on normal rabbits were available for all months of the year, the November and December counts were selected for analysis because in these months the largest samples were available for each hour of the day from 9 a.m. to 5 p.m. The variation in the formula of the blood between the 2 months was not significant.

The animal material consisted of 204 young adult male rabbits examined in the months of November and December in the years

---

* These studies were done at the Rockefeller Institute for Medical Research, in the laboratory of Dr. Wade H. Brown. They were carried out by the author, in coöperation with Drs. Louise Pearce, Paul D. Rosahn, and C. K. Hu. The technical assistance of Mr. Leslie Kish was invaluable.

[1] Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **51**, 83.

[2] Casey, A. E., Rosahn, P. D., Hu, C. K., and Pearce, L., *J. Exp. Med.*, 1936, **64**, 453.

1927-1933 inclusive. The age range was from 4 to 15 months and the average age was 8 months. All the animals were caged separately. The rabbits were usually alternated from week to week when repeated counts were made, so that counts are available for different times of the day on the same animal. In all, 963 counts (an average of about 5 per animal) were made, distributed as follows:

178 counts on 80 rabbits between 9 and 9:59 a.m.
226 " " 103 " " 10 " 10:59 "
193 " " 115 " " 11 " 11:59 "
30 " " 19 " " 12 noon and 12:59 p.m.
82 " " 60 " " 1 and 1:59 p.m.
124 " " 64 " " 2 " 2:59 "
85 " " 35 " " 3 " 3:59 "
45 " " 17 " " 4 " 4:59 "

In all, 597 determinations on 190 of the 204 rabbits were made between 9 a.m. and 12 noon, and 366 determinations on 136 of the 204 rabbits were made between 12 noon and 5 p.m.

The blood was collected in U. S. standardized Trenner pipettes. Acetic acid was employed as a diluent. The counts were made in a Levy-Hausser counting chamber. The counting technic was standardized, so that the average coefficient of variation due to technical error in a given total white count was 9%.

The material was analyzed in 2 periods, 1927-1929 inclusive, and 1930-1933 inclusive. In the first period the counts were made before the animals had been fed or permitted access to water, and only one white cell pipette was filled from each rabbit on a given day. In this period the material consisted of generally brown-gray hybrids purchased from dealers. In the second period the animals were permitted free access to water and food at all times. From 2 to 4 pipettes were filled at a single sitting and the results were averaged. In this period the material consisted for the most part of animals bred in the laboratory and of known genetic stock. Analysis on this basis afforded an opportunity for comparing results in animals of unknown hybrid stock without free access to food and water with results in animals of known genetic origin which had usually been in the laboratory for several generations and which were permitted free access to food and water.

Results. The mean values of the white blood cell counts for each hour of the day between 9 a.m. and 5 p.m. are presented in tabular form, together with the standard error of the mean. The variance between the means of the hours was not significantly different from the variance within the means. The white blood cells averaged 7,891 for the morning and 7,914 for the afternoon period, a difference of 23 ± 194 white blood cells (not significant).

TABLE I.
Hourly Means of Total White Blood Cell Counts in Normal Male Rabbits.

Hour	9:00 9:59	10:00 10:59	11:00 11:59	12:00* 1:59	2:00 2:59	3:00* 4:59	9-12 a.m.	12-5 p.m.
Counts	178	226	193	112	124	130	597	366
Animals	80	103	115	73	64	46	190	136
Mean	7709	7931	8033	8061	7945	7738	7891	7914
σ_m	±220	±195	±211	±276	±264	±255	±119	±152

*The data from 12 noon to 1:59 p.m. and from 3:00 to 4:59 p.m. were combined to give samples more comparable with those for the other hourly periods.

No single individual hourly mean differed from any other. Such variations as occurred could be explained on the basis of random sampling. There is therefore no evidence of a significant orderly variation in the total white blood cells of the rabbit in the hours between 9 a.m. and 5 p.m.

There is also no evidence in the rabbit that a digestive leukocytosis occurs between the hours of 9 a.m. and 5 p.m., as no statistically significant change was found in the group permitted free access to food and water.

Discussion. It has been observed that the lowest of repeated white blood cell counts in man are obtained early in the morning and the highest late in the afternoon.[3–14] The afternoon level is high even when food intake, muscular effort and emotional stress are controlled.[6, 7, 10–14] The controlled studies, however, are based on 12-50 counts made on the same day in the same persons, and the possible effect of the repetition cannot be discounted. It has been pointed out that successive painful punctures cause a successive rise in the leukocyte count,[12] and an analysis of the average hourly counts in the controlled series available on healthy individuals[10–14] reveals a

---

[3] Reinert, E., *Die Zählung der Blutkörperchen und ihre Bedeutung für die Diagnose und Therapie*, F. C. W. Vogel, Leipzig, 1891.

[4] Rieder, H., *Beiträge zur Kenntnis der Leukocytosis und verwändter Zustände des Blutes*, F. C. W. Vogel, Leipzig, 1892.

[5] Gregor, K., *Arch. f. Verdauungskrankh.*, 1898, **3**, 387.

[6] Japha, A., *Jahr. f. Kinderh.*, 1900, **52**, 242.

[7] Japha, A., *Deutsch. Aertz. Zeitung*, 1901, **3**, 145.

[8] Türk, W., *Vorlesungen über klinische Hämatologie*, W. Braumuler Vienna, 1912.

[9] Mauriac, P., and Cabouat, P., *Paris Méd.*, 1921, **11**, 407.

[10] Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwahl, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 14.

[11] Shaw, A. F. B., *J. Path. and Bact.*, 1927, **30**, 1.

[12] Garrey, W. E., *Proc. Staff Meet., Mayo Clin.*, 1929, **4**, 157.

[13] Smith, C., and McDowell, A. M., *Arch. Int. Med.*, 1929, **43**, 68.

[14] Kenyon, F., and Macy, I. G., *Human Biol.*, 1938, **10**, 511.

steady rise in the mean count until the series were variously discontinued at 4, 5, 7 and 8 p.m. The total rise for the 4 series in question averaged 2,330 ± 292 white blood cells (statistically significant). Most of the studies in which variations and rhythms were reported are based on an inadequate number of instances, and no statistical consideration of possible technical variations seems to have been undertaken.

The study herewith reported seems free from these disadvantages. The animal material was accustomed to existence in the laboratory and most of it was also accustomed to the taking of blood for examination. These circumstances perhaps lessened variations which might have been caused by bringing in animals to lead an unaccustomed laboratory existence and making the blood counts while they were in a state of digestive, environmental and emotional disturbance. In addition, only one count was made on a given animal during any given 3-day period. The data therefore seemed to rule out a possible diurnal variation in the total leukocyte count of the rabbit under controlled laboratory conditions.

When the material was examined with the idea of determining a possible bias for certain breeds counted at certain hours of the day, it was found that the breeds had been so varied that no bias of this kind was evident.

Summary and Conclusions. Nine hundred sixty-three total white blood cell counts made on 204 normal and apparently healthy young adult male rabbits between 9 a.m. and 5 p.m. revealed no statistically significant variation in the hourly means. The blood cell level, apart from sampling variations, was the same for each hour of the day. There was also no evidence of a digestive leukocytosis in the rabbit.

The mean value for 597 leukocyte counts on 190 of the 204 rabbits between 9 a.m. and 12 noon was 7,891, as compared with 7,914 for 366 counts on 136 of the 204 rabbits between 12 noon and 5 p.m. The difference (23 ± 194) was not significant.

These studies are significant for several reasons: The investigation covered a wide variety of breeds and hybrids. The animals were accustomed to laboratory existence and hemocytologic technic before the counts were made. Feeding conditions were varied during the study. Only one examination was made on a given animal on a given day, and usually not more than one or two counts were made on each animal per week. Finally, the material was analyzed by acceptable biometric procedures and represents by far the most extensive study of the sort which has been made.

11864

Thermo-Regulatory Function of Rat Scrotum. I. Normal Development and Effect of Castration.*

Frederick N. Andrews.† (Introduced by Fred F. McKenzie.)
From Missouri Agricultural Experiment Station and the United States Department of Agriculture coöperating.

It has been shown that the scrotum of certain mammals has a thermo-regulatory function,[1, 2] and that if the testes are experimentally placed within the abdominal cavity the germinal epithelium undergoes marked degeneration.[3, 4] In the ram, bull and boar the scrotum acquires the ability to contract and relax to temperature change as the testes begin the production of spermatozoa, and loses this ability following castration.[5] Further studies on the ram indicated that this function was apparently dependent upon the male hormone. Although the relations of temperature and contractility of the rat scrotum have not been investigated, Hamilton[6] reports that the rat scrotum depends upon male hormone for its development and maintenance and Wells[7] has demonstrated the dependence of the scrotal sac upon male hormone in the ground squirrel.

Procedure. The results of studies of the thermo-regulatory function of 7 normal and 6 castrate albino rat scrota are presented (age 9 weeks to maturity, Table I). Castration was performed at 5 weeks of age through a medial, abdominal incision. The contractility of the scrotum was measured by passing a thread attached to a recording lever through the skin on the ventral surface of the scrotum midway between the scrotal tip and the body; the rats were then anesthetized with nembutal and ether, securely fastened to a heavy animal board, and submerged, with the exception of the head, in a water bath. The temperature of the bath was decreased

---

*Contribution from the Department of Animal Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 700.

† The author wishes to express his appreciation to Dr. L. J. Wells of the Department of Anatomy, University of Minnesota, for his interest and advice in this study.

[1] Moore, C. R., and Quick, W. J., *Am. J. Physiol.*, 1924, **68**, 70.

[2] Phillips, R. W., and McKenzie, F. F., *Mo. Agr. Exp. Sta. Res. Bul.* 217, 1934.

[3] Griffiths, J., *J. Anat. and Physiol.*, 1893, **27**, 482.

[4] Moore, C. R., *Anat. Rec.*, 1922, **24**, 383.

[5] Phillips, R. W., and Andrews, F. N., *Mass. Agr. Exp. Sta. Bul.* 331, 1936.

[6] Hamilton, J. B., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 386.

[7] Wells, L. J., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 625.

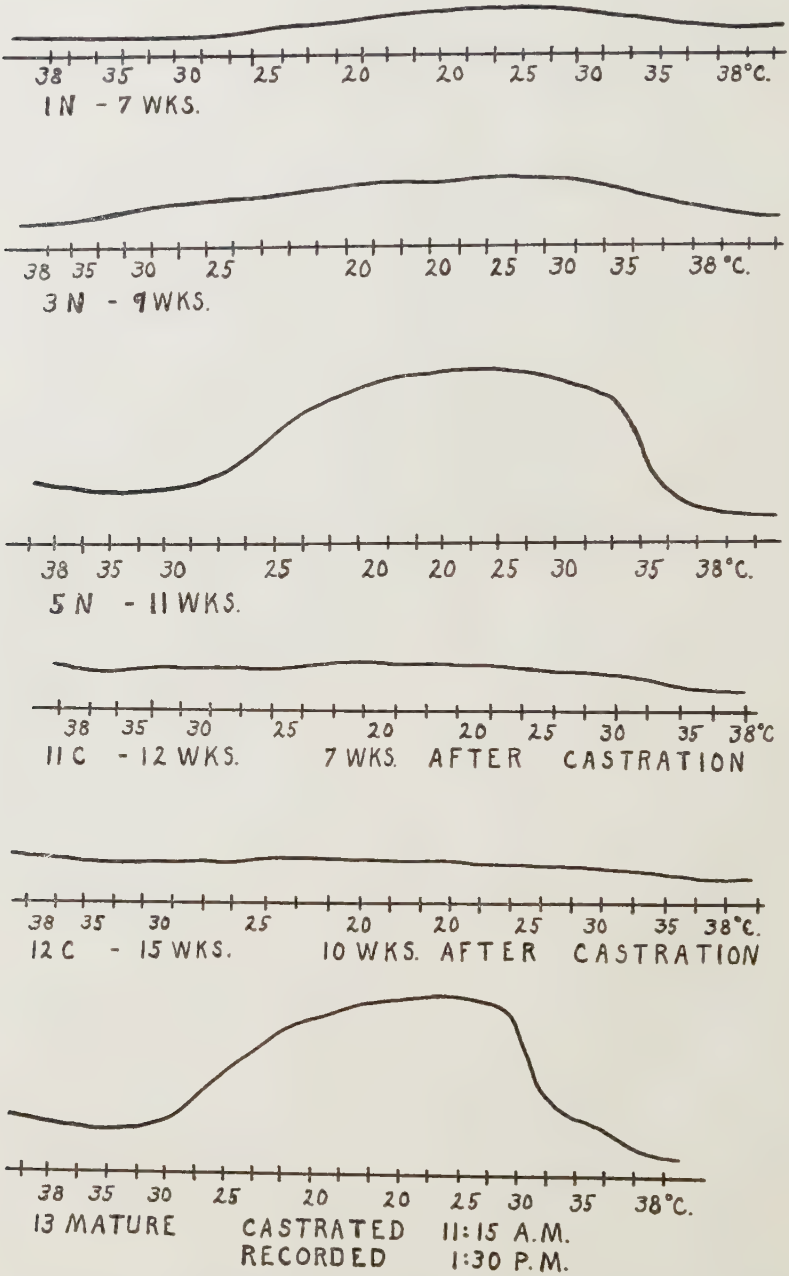


FIG. 1.

Tracings of kymograph records showing the effect of temperature change on the contractility of the rat scrotum. The time is recorded at two-minute intervals on the base line. N = Normal. C = Castrate.

TABLE I.
Development of Testis and Scrotal Sac in Normal Rats and of Scrotal Sac in Rats Castrated at 5 Weeks.

Rat No.	Age (wks)	Body wt at autopsy g	Testicular wt, g		Stage of spermatogenesis	Combined wt left and right scrotal sacs, g
			Left	Right		
1N	7	112	0.58	0.58	Sperm heads in some tubules	0.24
2N	8	141	0.76	0.72	” ” ” ” ”	0.27
3N	9	166	0.90	0.91	Abundant sperm	0.37
4N	10	191	1.10	1.17	Scattered ”	0.28
5N	11	220	1.21	1.37	Abundant ”	0.43
6N	12	226	1.40	1.43	” ”	0.42
7N	14	249	1.27	1.20	” ”	0.46
8C	6	96				0.11
9C	8	128				0.07
10C	10	184				0.11
11C	12	220				0.10
12C	15	227				0.18
13C	Mature	230				—

N = Normal. C = Castrate.

from 38°C to 20°C at the rate of about 1°C per minute and then raised to 38°C at the same rate. The scrotal movements were thus recorded on a kymograph. The rats were sacrificed immediately upon the completion of the kymograph records. The testes of the normal rats were removed, weighed fresh and fixed in Bouin's fluid, and the scrotal sacs (composed of the peritoneum, internal spermatic fascia, cremasteric skeletal muscle and external spermatic fascia[7]) were cut at the inguinal ring, weighed fresh, and fixed.

Results. Representative tracings of kymograph records showing the relation of temperature to scrotal contractility are shown in Fig. 1. The rapid testicular development initiated about the 9th week was followed by the ability of the normal rat scrotum to respond readily to temperature change. This ability did not develop in those rats castrated at 5 weeks of age. The presence of the testes in the scrotum is not essential as demonstrated by the fact that the fully developed scrotum of rat 13 responded readily although castration had been performed a few hours earlier. Testicular weights and changes, and scrotal sac weights are presented in Table I. The gradual loss of the thermo-regulatory function of the mature rat scrotum following castration, and the effects of cryptorchidism and male hormone upon scrotal contractility are now being studied in the hope that they may not only be of value in understanding the scrotal mechanism but of assistance in physiological assays of the male hormones.

11865 P

Cross-Excitation Between Mammalian Medullated Nerve Fibers After Treatment with Veratrine.

T. P. Feng and T. H. Li.

From the Department of Physiology, Peiping Union Medical College, Peiping.

Dun and Feng[1] have demonstrated with amphibian preparations that contrary to general belief nerve fibers, but more particularly motor nerve endings after treatment with veratrine, give prolonged repetitive discharges to a single stimulus. We have extended their work and found that mammalian motor nerve endings after veratrinization likewise show prolonged repetitive discharges which can be readily recorded from the ventral root. We have furthermore ascertained that in a cat which has received an intravenous injection of a suitable amount of veratrine, such as 2 cc of 0.1% solution, the nerve fibers in the nerve trunk can also show prolonged repetitive firing. In the course of our work we had occasion to make various control tests, some of which dealt with possibilities which at first seemed very remote. One of the tests consisted of stimulating one ventral root bundle and leading off from another ventral root bundle of the same segment and side, both bundles being sectioned centrally at their exit from the cord. In a normal cat, in view of Blair and Erlanger's work,[2] one would naturally expect to lead off nothing but stimulus artifact in this way; but in a veratrinized animal one could not be so sure. In fact we have found that in a veratrinized cat in which the motor nerve endings and the nerve fibers in the sciatic nerve trunk were giving prolonged abundant after-discharges, some discharges could generally be led off from one ventral root when another ventral root was stimulated with a single shock. Such discharges have characteristic long and rather irregular delays in their onset; their shortest latency being about 4 msec and the longest exceeding 30 msec. This long latency, in addition to other reasons, excludes the possibility that the discharges might be due to the escape of the stimulating current from one root to the other; and there is left only one reasonable interpretation that they result from cross-excitation between nerve fibers. The phenomenon appears not so strange, if one recalls that cross-excitation between amphibian medullated nerve fibers in the sciatic nerve trunk after treatment with hypertonic solutions has been shown by Kwassow and Naumenko.[3] The work of Jasper and Monnier[4] on the transmission of excitation between excised non-myelinated nerves may also be recalled here, and it is interesting to note that the transmission in this case is likewise attended by a long delay. For excitability changes in nerve fibers produced by impulses in adjacent nerve fibers see Otani[5] and Katz and Schmitt.[6] It is significant that in all cases so far known in which actual cross-excitation between nerve fibers takes place, the nerve fibers have a tendency to enter into rhythmical activity. This is true of the preparations used by Kwassow and Naumenko and by Jasper and Monnier; and it is also the case with our veratrinized mammalian nerve. An adequate discussion of the subject, however, can only be attempted later in the detailed report.

---

[1] Dun, F. T., and Feng, T. P., *Chinese J. Physiol.*, 1940, **15**, 405.

[2] Blair, E. H., and Erlanger, J., *Am. J. Physiol.*, 1932, **101**, 559.

11866

Effect of Foster-Nursing upon Inborn Resistance of Mice to St. Louis Encephalitis.

F. HOWELL WRIGHT. (Introduced by Leslie T. Webster.)

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

Previous studies from this laboratory have demonstrated that certain factors which regulate the resistance of mice to infectious disease are inherited.[1] Bittner's recent work, however, on the influence of mothers' milk on the susceptibility of mice to cancer indicates that the same sort of foster-feeding test should be applied to the problem of susceptibility to infection.[2]

Litters inherently susceptible to St. Louis encephalitis were placed with mothers of resistant litters and vice versa within 24 hours of birth. As controls, susceptible litters were likewise interchanged among susceptible mothers and resistant litters among resistant mothers respectively. Foster-nursing was continued 3 weeks and then each mouse of each litter was inoculated intranasally with 0.03 cc of a 1:10 or 1:100 suspension of mouse-brain St. Louis encephalitis virus. Further controls from litters nursed by their own mothers were likewise inoculated.

---

[3] Kwassow, D. G., and Naumenko, A. I., *Pflugers' Arch.*, 1936, **237**, 576.

[4] Jasper, H. H., and Monnier, A. M., *J. cell.-comp. Physiol.*, 1938, **11**, 259.

[5] Otani, T., *Japanese J. Med. Sciences: Biophysics*, 1937, **4**, 355.

[6] Katz, B., and Schmitt, O. H., *J. Physiol.*, 1940, **97**, 471.

[1] Webster, L. T., *J. Exp. Med.*, 1937, **65**, 261.

[2] Bittner, J. J., *Am. J. Cancer*, 1940, **39**, 104.

The results of the tests are shown in Fig. 1. Of 104 susceptible young mice nursed by their own mothers, 90% succumbed between the 5th and 7th days and 96.2% by the end of the 3-week period of observation (Fig. 1-A). Foster-nursing of 65 susceptibles

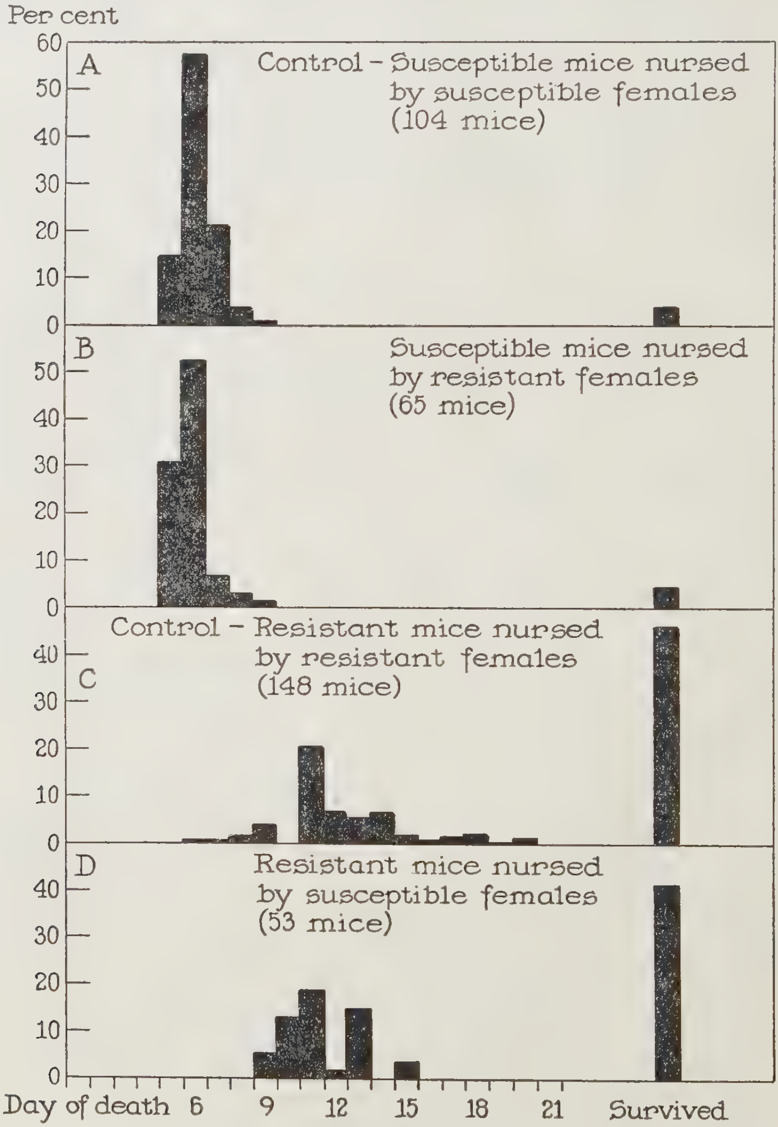


Fig. 1.

Period of survival of control and foster-nursed mice of the virus-resistant and virus-susceptible strains.

by resistant foster mothers gave almost identical results (Fig. 1-B). Of 148 resistant young mice nursed by their own mothers, approximately 54% succumbed (Fig. 1-C), a figure which is higher than that previously reported and due, in all probability, to the discontinuance of selective breeding for 3 years. In contrast to the susceptible mice, the resistants rarely died before the 9th day, at which time nearly all the susceptibles were dead, and most frequently on or about the 11th day. Foster-nursing of 53 resistant young by susceptible mothers again produced no change in the amount or distribution of deaths (Fig. 1-D).

Summary. In strains of mice selectively bred for resistance or susceptibility to St. Louis encephalitis virus, foster-nursing exerted no effect upon the course of disease or mortality-rate following nasal instillation of virus.

11867 P

Secretion of Inulin by the Kidney of the Crayfish.*

N. S. RUSTUM MALUF. (Introduced by E. C. Faust.)

From the Department of Zoology, The Johns Hopkins University, Baltimore, and the Department of Tropical Medicine, The Tulane University, New Orleans.

Cumulative evidence indicates that inulin is not secreted by nor passively reabsorbed through the vertebrate nephric tubule (Smith[1]). In vertebrates, the inulin-clearance, therefore, is independent of the concentration of inulin in the plasma and is used as a measure of the rate of glomerular filtration.

In an attempt to find whether filtration occurs through the nephron of the crayfish by the use of inulin, the writer obtained most unexpected results as regards the inulin-clearance. This is the first invertebrate kidney which has been studied from the standpoint of "clearances". Inulin was measured by a micro-adaptation of the Shaffer-Hartmann-Somogyi method.

Nowhere in the crayfish nephron[2] is there a tenuous syncytium such as constitutes the glomerular capsule of the vertebrate nephron.

---

* This work was performed largely during the tenure of a Johnston Research Scholarship at The Johns Hopkins University. The measurements of inulin-clearance were completed in the Department of Tropical Medicine, Tulane University.

[1] Smith, H. W., *The Physiology of the Kidney,* New York, Oxford University Press, 1937.

[2] Maluf, N. S. R., *Zool. Jb., Abt. f. allg. Zool. u. Physiol. d. Tiere,* 1939, **59**, 515.

The renal arterioles open freely into the cavernous haemocoele which follows the numerous involutions of the renal epithelium. The kidneys of the crayfish consist of a pair of relatively large nephrons, each of which ends blindly in a highly lobulated coelomosac.

Contrary to the condition in the vertebrate aglomerular kidney, inulin, xylose, and glucose appear in the urine of the crayfish after being injected into the blood stream. This, however, is not proof of filtration because the inulin-clearance is not independent of the concentration of inulin in the plasma. The inulin-clearance : plasma-inulin curve (Fig. 1), which resembles an adsorption isotherm, shows that inulin is outwardly secreted. This is further indicated by the fact that the shape of the curve in Fig. 1 is determined practically entirely by the urine/plasma ratio for inulin (Fig. 2).

The sudden decline in the inulin-clearance with augmentation of the plasma-inulin (Fig. 1) indicates that inulin is not appreciably filtered. Filtration, therefore, apparently does not occur through the crayfish nephron.

Since the labyrinthal portion of the crayfish nephron is the only tissue of the body which is capable of accumulating and outwardly secreting a number of dyes (cyanol, phenol red, indigo disulfonate, acid and basic fuchsin),[3] it seems probable that inulin, xylose, and

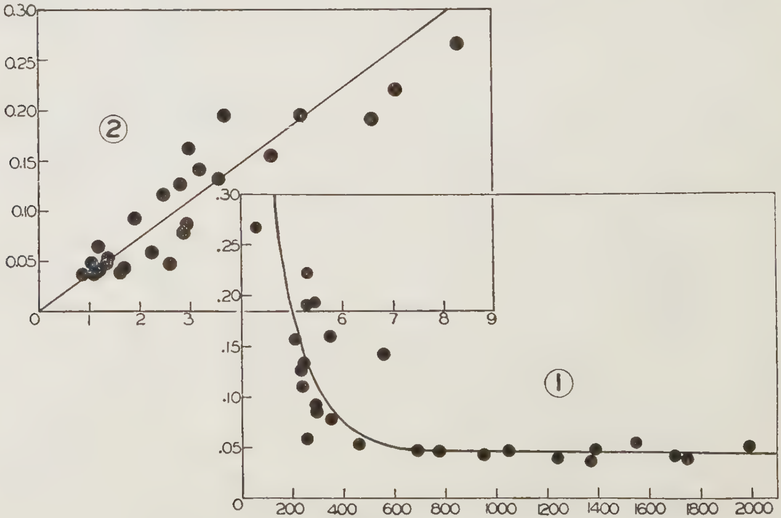


FIG. 1.

The inulin-clearance (ordinate) in cc per hour as a function of the plasma-inulin concentration (abscissa). Each point stands for a single separate crayfish.

FIG. 2.

The urine/plasma ratio for inulin (ordinate) as a function of the plasma-inulin concentration (abscissa). Each point stands for a single separate crayfish; the same individuals as in Fig. 1.

---

[3] Unpublished data.

glucose are outwardly secreted by the labyrinth. In contrast to the condition in the aglomerular nephron of vertebrates, cyanol, a blue anilin dye, is outwardly and rapidly secreted by the crayfish kidney even when present in low concentrations in the plasma. Evidently the labyrinth has varied secreting capacities.

The maintenance of an internal aqueous and saline steady state, in spite of a constant inward diffusion of water, is known to be due to the unvarying capacity of the crayfish nephron to eliminate urine that is markedly hypotonic to the blood. Since filtration apparently does not occur through this nephron, the hypotonicity cannot be explained by a reabsorption of salts but, like the aglomerular teleost kidney, is evidently due to a secretion of water.

The above facts permit the following conclusions with regard to the physiology of the nephron of the crayfish: 1. Inulin is secreted. 2. Filtration is evidently not an appreciable factor in urine-formation. 3. The manufacture of a hypotonic urine must be due to a secretion of water.

11868 P

Treatment of Cecal and Liver Trichomoniasis in Turkeys by Fever Therapy.

MARLOW W. OLSEN AND ENA A. ALLEN. (Introduced by R. M. Fraps.)

From the Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Since 1937, experimental work has been in progress on the use of fever therapy for the cure of cecal and liver trichomoniasis in turkeys, a widespread disease of domestic fowls caused by the protozoan parasite, *Trichomonas gallinarum*. This organism invades the tissues of the lower digestive tract of birds and in chronic infection produces lesions in the ceca and liver. These lesions closely resemble, and undoubtedly have been often confused with, those produced by *Histomonas meleagridis* in the disease commonly known as blackhead. Birds with chronic trichomoniasis are droopy in appearance, refuse to eat, and lose weight. The droppings become quite liquid and light yellow in color. Young birds usually die 4 to 8 days after exhibiting the first symptoms of the disease, although in mature birds the period between the first symptoms and death may be much longer.

Most of the turkeys used in this preliminary study were naturally infected and ranged from 3 to 18 months in age. At the beginning of the work the disease was diagnosed by the external symptoms noted above, and by microscopic examination of the cecal discharges.

Recently it has been found that by making an incision into the abdominal cavity, and with the aid of a strong light, it is possible to observe the liver and ceca directly for lesions. The direct observation of lesions and identification of trichomonads in cecal droppings have made certain the elimination from treatment of birds exhibiting external symptoms similar to those of trichomoniasis but actually due to other causes.

Birds undergoing treatment were placed in a thermostatically controlled cabinet for periods ranging from 1 to 2 hours. The internal body temperature of the bird was raised from 2 to 6 degrees above the normal of 106.5°F by maintaining an air temperature within the cabinet of approximately 104°F, and a relative humidity of 60 to 70%. The duration of treatment depended upon the physical condition of each bird and its ability to withstand the increased temperature. Usually three treatments at intervals of every other day were sufficient to check the disease, although as many as 6 treatments were necessary in a few more advanced cases. After treatment the birds were placed in wire-bottomed cages at room temperature.

Heat treatment was administered to 19 turkeys in which trichomoniasis was diagnosed by external symptoms and microscopic examination and to 5 turkeys in which liver and cecal lesions were directly observed. Twenty of these 24 treated birds recovered, while 4 failed to respond to treatment. Those birds which recovered usually showed signs of improvement after the second or third treatments, began to eat voluntarily, gained in weight, and behaved like normal, active birds. Four adult turkeys were killed at different stages of recovery following treatment, and postmortem examination disclosed that many of the liver lesions had almost disappeared and all others were in process of healing. Cultures made from the necrotic areas of these livers were completely negative for trichomonads. Of the 4 birds which failed to respond to the treatment, 2 died during or shortly after being given the first treatment.

Nine turkeys with trichomoniasis were kept in cages similar to, and fed rations identical with, those of the treated birds. Seven of these died as a result of the disease, and trichomonads were cultured from their livers and ceca, while the remaining 2 birds recovered. These results indicate that turkeys having trichomoniasis of the lower digestive tract can be effectively treated with fever therapy. Work is being carried out with experimentally inoculated birds.

AUTHORS' INDEX

(*The numerals indicate the page.*)

Abrams, A. 46.
Abramson, D. I. Hypertension, blood flow, 127.
Adler, H. F. 98.
Adriani, J., Martin, S. J., and Rovenstine, E. A. Chromodacryorrhea, cyclopropane, 785; Anesthesia, spinal, venous pressure, 415.
Aird, R. B., Cohn, W. E., Weiss, S. Triphenyl phosphite, toxicity, 306.
Alberts, E. 563.
Almquist, H. J., Klose, A. A. Antihemorrhagic compounds, 55; and Zander, D. Chick diet, charcoal, 303.
Althausen, T. L. 405.
Anderson, E., Herring, V., and Joseph, M. Adrenalectomy, cbhy. stores and NaCl, 488.
Anderson, R. S. 713.
Andrews, F. N. Scrotum, development, castration, 867.
Antopol, W. 15, 447.
Auchincloss, R. 820.
Axtmayer, J. H. 583.

Bachrach, W. H. 66.
Badger, E. 377.
Bailey, P., and Haynes, W. Respiratory center localization, 686.
Barach, A. L., and Steiner, A. Electrocardiogram, O_2 and CO_2 inhalation, 175.
Barnes, R. H., and MacKay, E. M. Vitamin B_1 deficiency, insulin, 759.
Barr, J. S. 539.
Bartels, W. E. 394, 397.
Bartlett, W., Jr. Hyperthyroidism, alkali reserve, acid excretion, 196.
Bassett, A. M., Althausen, T. L., and Coltrin, G. Jaundice test, 405.
Bassett, L. 272.
Becker, R. F. Development, embryonic, 689.
Beckman, H., and Ota, R. K. Plasmodium cathemerium, sporozoites, 477.
Belasco, I. J. 706.
Ben-Dor, B. Dermatitis in chickens, 495.
Berman, A. L., and Ivy, J. H. Cinchophen, excretion in bile, 853.
Bernkopf, H., and Kligler, I. J. Virus, rabies, properties, 332.
Bernstein, S. S. 151.
Bethlehmy, S. 317.
Beutner, K. R. 337.
Beutner, R., and Beutner, K. R. Ethylaminobenzoate, anesthetic, 337.
Bittner, J. J. Cancer, mammary, milk, 805.
Blackfan, K. D. 383.
Blair, A. P., Hargreaves, C. C., and Chen, K. K. Ouabain, cymarin, coumingine HCl, susceptibility, 209.
Block, R. J., Bolling, D., and Kondritzer, A. A. Microestn. leucine, isoleucine, valine, 289.
Blumberg, H., and Gardner, R. E. Gastric adenoma, *Cysticercus fasciolaris,* 673.
Boatner, C. H., and Efron, B. G. Antigen purification, grass pollen, 460; and Pabst, M. R. Antigens, ragweed pollen, 637.
Bohls, S. W., Irons, J. V., and De Shazo, T. Relapsing fever spirochetes, cultivation, 375.
Bolling, D. 289.
Booth, A. N. 769.
Bourdillon, J. Virus, influenza, electrophoresis, 679.
Bowman, D. E., Morris, L. E., and Stacy, J. R. Ascorbic acid, hypophysectomy, 784.
Bowman, K. M. 468.
Boyd, E. M., Clarke, M. E., Clarke, E. L., and Segal, A. L. Post. pituitary, H_2O retention, 20.
Boyle, P. E. 298.
Bradel, S. 104.
Bradley, W. B., and Ivy, A. C. Cinchophen, detn. in bile, 143; 66.
Brewer, G. Plasma K, epinephrine, liver, 266.
Bricker, E. M. 710.
Brown, R. Pneumococcus, pptn. of specific substances, 93.
Buchanan, A. R. Circling, brain stem lesions, 389.
Buddingh, G. J. 546.
Burnham, L. 346.
Burrill, M. W. 452; 780.
Burris, R. H., and Wilson, P. W. Respiratory activity of cells, 721.

Cadden, J. F. Canavanine, prepn., structure, 224.
Cahn-Bronner, C. E. Bacteria, O_2 needs, 454.
Cantarow, A., Wirts, C. W., Jr., and Hollander, G. Serum bilirubin, 253.
Carden, G. A., Province, W. D., and Ferrebee, J. W. Vitamin B_1, excretion, 1.
Carnes, W. H. Adrenal, fetal, androgens, 502.
Casey, A. E. Blood leucocytes, diurnal levels, 863.
Chadwick, C. S. Prolactin, water drive factor, 335.
Cheever, F. S. Carcinoma, Brown-Pearce, antibody, 517.
Chen, C. Y. 78.
Chen, G., Oldham, F. K., and Geiling, E. M. K. Pituitary melanophore hormone, chick embryo, 810.
Chen, K. K. 209.
Chen, K. Clostridium tetani, cultivation, 826.
Cheney, G. Ulcer, gastric, diet and cinchophen, 190.
Chunn, C. F., and Harkins, H. N. Blood, alimentary absorption, 569.
Churg, J. 15, 447.
Clark, P. F. 232.
Clarke, E. L. 20.
Clarke, M. E. 20.
Claugus, C. E. 256.
Clowes, G. H. A. 719.
Cohen, P. P. 576.
Cohen, S., and Mueller, J. H. *C. diphtheriae,* oleic acid, 244.
Cohn, W. E. 306.
Collings, W. D. 470.
Coltrin, G. 405.
Conti, O. 493.
Cooper, M. L., and Keller, H. M. Sulfonamides, *Shigella paradysenteriae,* 111.
Coulson, E. J., and Stevens, H. Ragweed extract, sensitization, 798.
Cutuly, E. Hormones, implantation, 659.

Dalton, A. J., and Hanzal, R. F. Chick embryo, cbhy. metabolism, 278.
Daly, C. 468.
Darrow, C. W. Electrode, encephalogram, 301.
David, N. A. 667.
Davis, J. E. (Blood) Polycythemia, cobalt, 671.
Davis, M. W., Bradley, W. B., Bachrach, W. H., and Ivy, A. C. Ulcer, cinchophen, 66.
Day, H. G., and McCollum, E. V. Zinc deficiency, 282.
Dean, R. B. Muscle, K uptake, 817.
De Eds, F. Phenothiazine, oxidation, 632.

DeGraff, A. C., and Lehman, R. A. Lanatoside C, digoxin and digoxigenin pharmacology, 323.
De Shazo, T. 375.
Deuel, H. J., Jr. 96.
Diamond, L. K. 383.
Diaz, J. T. Estrous cycle, nephrectomy, 226.
Dinnean, F. L. 183.
Dorfman, A., Rice, L., Koser, S. A., and Saunders, F. Sulfonamides, dysentery respiration, 750.
Dorfman, R. 62.
Dorfman, R. I. Estrogens, comparison, 594. Testosterone, metabolism, 739.
Doster-Virtue, M. E., and Virtue, R. W. Intestinal loops, preparation, 813.
Doubly, J. A. 801.
Dow, P., and Wiggers, C. J. Recovery from fibrillation, 355.
Dragstedt, C. A. 688.
Dubos, R. J. 462.
Duerschner, D. R. 556.
Duran-Reynals, F., and Estrada, E. Protection against Rous sarcoma, 367.

Eaton, M. D. 677; and Nicewonger, C. R. Influenza antigen, antibody, 439.
Ecker, E. E. 130; Pillemer, L., and Kuehn, A. O. Complement, thermostability, 115.
Edmondson, H. A., and Fields, I. A. Pancreas necrosis, Ca, 803.
Efron, B. G. 460, 637.
Ellinger, F. Roentgen-pigmentation, 148.
Ellison, F. S. 181.
Elvehjem, C. A. 482; 769.
Emerson, G. A. 135, 157; and Evans, H. M. Vitamin E-low rats, 159.
Emery, E. S., Jr., 475.
Engel, R. W. 621.
Ensworth, H. K. 734.
Eppstein, S. E., and Morgulis, S. Alpha-tocopherol, daily need, 715.
Erf, L. A., and Pecher, C. Strontium in milk, 762; Tuttle, L. W., and Scott, K. G. Phosphorus, radio, retention, 652.
Erickson, B. N. 151; and Ferguson, J. H. Blood clot, phospholipids, 579; Souders, H. J., Shepherd, M. L., Teague, D. M., and Williams, H. H. Blood elements, cerebroside, neutral fats, 153.
Estrada, E. 367.
Evans, H. M. 135, 157, 159, 627.

Fan, C., and Woo, T. T. Vitamin C, creatin metabolism, 90.
Fazekas, J. F. 468.
Feinstein, R. N., and Stare, F. J. Tumor tissue oxidation, 525; and Stare, F. J. Glutathione, protein oxidation, 529.
Fekete, E. 796.
Feng, T. P., and Li, T. H. Nerves, cross-excitation, veratrine, 870.
Ferguson, J. H. 43, 579.
Ferguson, L. W. 120.
Ferrebee, J. W. 1.
Fields, I. A. 803.
Findley, T., and White, H. L. Kidney, diodrast, inulin clearances, 623.
Finn, S. B., and Kramer, M. Rickets, fluorine, 843.
Flippin, H. F. 317.
Ford, M. L., Petersilge, C. L., Young, A. F., and Wiggers, C. J. Anoxia, economy effort index, 353.
Forkner, C. E. 556.
Foster, C., Jones, J. H., Russell, D., and Dorfman, R. Grass culture, soilless, 62.
Foster, R. H. K. Antihemorrhagic comp., pharmacology, 412; 407.
Fraenkel-Conrat, H. L., Simpson, M. E., and Evans, H. M. Pituitary, ant., follicular hormone, 627.
Francis, T., Jr. Influenza, differentiation, 861.
Freeman, G. Hypertension, antipressor effect of blood, 185.
Friedewald, W. F., and Anderson, R. S. Virus, rabbit papilloma, inactivation, 713.
Friend, D. G. Tissue metabolism, drugs, 140; and Hastings, A. B. Tissue metabolism, detn., 137.
Frobisher, M., Jr., and Parsons, E. I. Diphtheria toxin, susceptibility, 165.
Fugo, N. W. 10.

Galapeaux, E. A. 98.
Gall, E. A., Barr, J. S., and Lingley, J. R. Phosphorus, effect on epiphysis, 539.
Gardner, R. E. 673.
Gardner, W. U. Estrogen, femur strength, 230, 287; Mamma, hypophysectomy, 835.
Geiling, E. M. K. 810.
Geist, S. H. 522; 766.
Gelarie, A. J. Chick embryo, development, 351.
Gilson, A. S., and Mills, W. B. Muscle, single response, 650.
Glazke, A. J., and Ferguson, J. H. Tryptase, heparin inhibition, 43.
Goldfarb, W. 468.
Gorbman, A. Ant. pituitary, thyrotropic, assay, 772.
Goss, H. 717.
Gotzl, F. R., and Dragstedt, C. A. Thyroid, tissue histamine, 688.
Grace, A. W., Rake, G., and Shaffer, M. F. Lymphogranuloma venereum, Frei test, 259.
Grady, H. G. 246.
Greene, R. R. 452; and Burrill, M. W. Androgen, response, 780; and Harris, S. C. Androgens, uterine wt., 34.
Greslin, J. 311.
Griffith, W. H., and Mulford, D. J. Methylation, choline, creatine, 657.
Grobstein, C. Testosterone, anal fin regeneration, 484.
Gyorgy, P., and Poling, C. E. Achromotrichia, 773.

Haag, H. B., and Taliaferro, I. Ascorbic acid, colon, 479.
Haagensen, C. D., Randall, H. T., and Auchincloss, R. Cancer, mammary, thyrotropic hormone, 820.
Hac, L. R. *N. gonorrhoeae*, culture, 381.
Haerem, A. T. Sulfonamide, tissue response, 536.
Hageman, P. O., and Doubly, J. A. *Brucella abortus*, immune response, 801.
Hamilton, H. L. Melanophore, steroids, 571.
Hammett, F. S. Tumor incidence, proline, 601.
Hammon, W. M. Poliomyelitis virus propagation, 124.
Hanzal, R. F. 278.
Hardy, J. D. 397.
Hargreaves, C. C. 209.
Harris, S. C. 34.
Harkins, H. N. 569.
Harrow, B. 46.
Hart, E. B. 769.
Harvey, S. C. 647.
Hastings, A. B. 137.
Hause, W. A. 387.
Haynes, W. 686.
Hays, H. W. 470.
Herring, V. 488.
Himwich, H. E., Bowman, K. M., Daly, C., Fazekas, J. F., Wortis, J., and Goldfarb, W. Insulin hypoglycemia, cerebral blood flow, 468.
Hinrichs, M. A. Circulation, peripheral, fatigue, 685.

Hirschboeck, J. S. Blood coagulation, collodion lined vessels, 122.
Hirschmann, D. J. 729.
Hoagland, C. L., Ward, S. M., Smadel, J. E., and Rivers, T. M. Vaccinia, biotin, 669.
Hodge, H. C. 70, 424.
Hoff, H. E., and Nahum, L. H. Heart, action current, KCl, 263.
Hollander, G. 253.
Holmes, R. O. 267.
Honig, C. D. 214.
Hook, W. E. Brown fat extr., metabolism, 37.
Hooker, C. W. Progesterone, uterus, 270.
Hornibrook, J. W. Nicotinic acid, growth, 598.
Hoskins, W. 72.
Hoskins, W. H. 60.
Hotchkiss, R. D. 462.
Howe, P. R., Wesson, L. G., Boyle, P. E., and Wolbach, S. B. Rickets, low Ca, 298.
Huang, C. H., and Sia, R. H. P. Streptococcus, virulence tests, 87.
Hudson, N. P. Virus, Mexican typhus, protection, 40; 361.
Humfeld, H. 729.
Hunter, F. E. 424.

Iglesias, R. 788.
Ingle, D. J., and Kendall, E. C. Adrenal cortex, muscle efficiency, 602.
Irons, J. V. 375.
Isaacs, R. Pernicious anemia-like syndrome, 794.
Ivy, A. C. 66, 143, 174, 313, 373.
Ivy, J. H. 853.

Jailer, J. W., and Leathem, J. H. Antigonadotropic subst., pregnant mare serum, 506.
Jewell, M. L. 169.
Jimenez, J. F. Carrion's disease, cultivation, 402; and Buddingh, G. J. Carrion's disease, 546.
Johnson, G. *Trichomonas vaginalis*, pH, 567.
Jones, L. R. 31.
Jones, J. H. 62.
Joseph, M. 488.
Joy, H. H. Sulfonamides, *B. pyocyaneus*, 709.
Jukes, T. H. Reproduction and vitamin B., 625; 296.

Kaminester, S. 749.
Kao, H. C., and Sherman, H. C. Vitamin A of tissues, 589.
Katzin, E. M. 343, 346.
Keith, T. B. 50.
Keller, H. M. 111.
Keltch, A. K. 719.
Kemp, C. R. 427.
Kendall, E. C. 602.
Kessel, J. F., and Stimpert, F. D. Poliomyelitis virus transmission, 665.
Kesten, H. D. 543.
Kirschbaum, A., Strong, L. C., and Gardner, W. U. Leukemia, methylcholanthrene, 287.
Kirstein, M. B. 640.
Kleiber, M., and Smith, A. H. Metabolism, fasting, 630.
Kleinman, A. 25.
Kligler, I. J. 332.
Klose, A. A. 55.
Klosterman, J. A. 464.
Knoll, A. F. Anaphylaxis, histaminase, 606.
Knott, E. M. Vitamin B_1 infant requirement, 765.
Kondritzer, A. A. 289.
Koser, S. A. 750.
Koster, H. Peritonitis, prevention, 660.
Krahl, M. E., Keltch, A. K., and Clowes, G. H. A. Flavin-dinucleotide, Arbacia ova, 719.
Krakower, C., and Axtmayer, J. H. Tocopherol, muscle lesions, 583.
Kramer, M. 843.
Krause, R. F. 424.
Krichesky, B. 431; and Honig, C. D. Uterine extr., toxicity after estrogen, 214.
Kubicek, W. G. 831.
Kuehn, A. O. 115.
Kupferberg, A. B. *Trichomonas vaginalis*, cell size, pH, 220.
Kurotchkin, T. J., Van der Scheer, J., and Wyckoff, R. W. G. Sera, rickettsial, refined, 323.

Laborde, J. C. 493.
Lackey, R. W. 8.
Lawson, H. Oncometry, 5.
Lazarus, A. S. B. typhoid, typing, 400.
Levine, P., and Katzin, E. M. Pregnancy, isoimmunization, 343; Katzin, E. M., and Burnham, L. Isoagglutinins, atypical, 346.
Leathem, J. H. Prospermin, pituitary, 497; 506.
Lee, J., Solmssen, U. V., Steyermark, A., and Foster, R. H. K. Antihemorrhagic compd., 407.
Lehman, R. A. 323.
Lehr, D., Churg, J., and Antopol, W. Liver, kidney damage by Na sulfamethylthiazole, 447; Antopol, W., Churg, J., and Sprinz, H. Sulfonamides, Na salts, toxicity, 15.
Leser, A. J., and Thienes, C. H. Procaine and atropine, 109; Thienes, C. H., and Tyler, D. B. Body temperature, drug toxicity, 682.
Li, T. H. 870.
Lightbody, H. D., and Kleinman, A. Arginase, liver, fasting, 25.
Lingley, J. R. 539.
Lipschutz, A., Iglesias, R., and Vargas, L. Estrogen, fibroids, 788.
Little, C. C. 796.
Little, R. B., Dubos, R. J., and Hotchkiss, R. D. Streptococci, gramicidin, 462.
Liu, W., and Zia, S. H. Typhus rickettsia, 823.
Loeb, E. N. 248.
Lustok, M. J. 576.

McCarter, J. R. 357.
McCarty, M. A. 50.
McCollum, E. V. 282.
McCune, D. J., and Weech, A. A. Phosphorus, determination, 559.
McOmie, W. A. 614.
McElroy, L. W., and Goss, H. Vitamin B_6 deficiency, cure, 717; and Jukes, T. H. Biotin, formation in rumen, 296.
McGee, L. C., and Emery, E. S., Jr. Amino acids, absorption, 475.
McIver, M. A. Chloroform poisoning, thyroxin, 201.
McNaught, J. B., and Zapata, E. M. *Trichinella spiralis*, incidence, 701.
MacLachlan, P. L. 392.
MacKay, E. M. Kidney hypertrophy, testosterone, 216; 437; 759.
Macy, I. G. 151.
Magill, T. P. Virus, influenza-like, 162.
Main, R. J. Epinephrine, respiration, B.P., 776; valve, mercury, 207.
Maluf, N. S. Insulin, crayfish kidney, 873.
Manly, M. L., Hodge, H. C., and VanVoorhis, S. N. P distribution in bone, teeth, 70.
Maresh, F., Lustok, M. J., and Cohen, P. P. Rhenium, physiology, 576.
Martin, S. J. 785.

Meeker, D. R., and Kesten, H. D. Atherosclerosis, high protein diet, 543.
Melampy, R. M., and Olsan, R. D. Bee, carbohydrates, 754.
Mettler, C. C. 473.
Mettler, F. A., Mettler, C. C. Caudate, labyrinthine disregard, 473.
Milhorat, A. T., Bartels, W. E., and Toscani, V. Vitamin C excretion, liver injury, 394; Hardy, J. D., Bartels, W. E., and Toscani, V. Vitamin C, creatine excretion, shivering, iodoacetate, epinephrine, 397.
Miller, B. F., Muntz, J. A., and Bradel, S. Dental plaque metabolism, detergent, 104.
Miller, F. R., and Hause, W. A. Specific subst. from leukemia urine, 387.
Miller, R. C., Keith, T. B., McCarty, M. A., and Thorp, W. T. S. Pig lameness, Mn, 50; and Shaw, J. H., Elvehjem, C. A., and Phillips, P. H. Adrenal necrosis, pantothenic acid, 482.
Mills, W. B. 650.
Minnich, V., Wright, W. T., Moore, C. V., and Spies, T. D. Blood ascorbic acid in pellagra, 441.
Mitchell, H. K. 565.
Molloy, E. 327.
Moore, C. V. 441.
Morales-Otero, P. 509; and Pomales-Lebron, A. Sulfonamides, Brucella, 512.
Morehouse, M. G., and Deuel, H. J., Jr. Caproic acids, metabolism, 96.
Morgulis, S. 715.
Morris, L. E. 784.
Mueller, J. H. 243, 244.
Muir, R. D., Shamleffer, V., and Jones, L. R. Sulfonamides, enteritidis, 31.
Mulford, D. J. 657.
Mullins, L. J. Radioactive ion distribution, 856.
Muntz, J. A. 104.
Muschenheim, C., Forkner, C. E., and Duerschner, D. R. Sulfonamides, tuberculosis, 556.
Mussio Fournier, J. C., Conti, O., and Laborde, J. C. Melanophore hormone, 493.

Nahum, L. H. 263.
Nelson, M. M., Emerson, G. A., and Evans, H. M. Tocopherol, growth, 157.
Nelson, V. E. 792.
Neuwirth, I., Klosterman, J. A. Lactic acid, mouth, 464.
Nicewonger, C. R. 439.
Noble, T. A., Wigodsky, H. S., and Ivy, A. C. Erythrocytes, gastric juice, 373.

Ogden, E., and Sapirstein, L. A. Pitressin, activation, 573
Oldham, F. K. 810.
Olitsky, P. K. Virus, Theiler's, immunology, 339.
Olsan, R. D. 754.
O'Neill, J. F. 782.
Oppenheimer, M. J., and Spiegel, E. A. Conditioned reactions, cortical lesions, 418.
Osgood, E. E. Leukemic cells, irradiation, 131.
Ota, R. K. 477.
Ouyang, G. 83; 845.

Pabst, M. R. 637.
Pang, K. H. Meningococcus, egg yolk, 848; and Zia, S. H. Typhus culture, 76.
Pappenheimer, A. M. Myopathy prevention, α-tocopherol, 457.
Parsons, E. I. 165.
Paul, W. D., and Kemp, C. R. Serum electrolytes, hyperpyrexia, 427.
Pearson, H. E., and Eaton, M. D. Pneumonia, virus, 677.
Pecher, C. 762.
Penrod, K. E. 222.
Persike, E. C., Jr. Thymus, pregnancy involution, 315.
Petersilge, C. L. 353.
Phatak, N. M., Zener, F. B., and David, N. A. Pregnancy, I therapy and B. M. R., 667.
Phibbs, B. P., Wigodsky, H. S., and Ivy, A. C. Bile, bile salt effect, 313.
Phillips, P. H. 482.
Pillemer, L. 115; Seifter, S., and Ecker, E. E. Complement, fat solvents, 130.
Pinkerton, H., and Swank, R. L. Virus, psittacosis, thiamin deficiency, 704.
Plummer, N., and Ensworth, H. K. Sulfonamides, excretion, 734.
Pohlman, A. G. Bone conduction, sensitivity, 662.
Poling, C. E. 773.
Pollock, W. 431.
Pomales-Lebron, A. 512. and Morales-Otero, P. Streptococci, hemolytic, 509.
Ponder, E. 617.
Porter, P. M. Blood, erythrocyte hemolysis, 744.
Province, W. D. 1.

Rafferty, M. A., and MacLachlan, P. L. Blood sugar, temperature, 392.
Rake, G. 259.
Rammelkamp, C. H., and Jewell, M. L. Sulfonamides, typhoid-dysentery group, 169.
Randall, H. T. 820.
Rasmussen, A. F., Jr., and Clark, P. F. Poliomyelitis, susceptibility, 232.
Rea, C. 54.
Reames, H. R. 838.
Reeder, W., and Nelson, V. E. Fluorescence. protein, 792.
Reinhardt, W. O., and Holmes, R. O. Adrenalectomy, lymphoid tissues, 267.
Reinhold, J. G., Schwartz, L., Flippin, H. F., and Bethlehmy, S. Sulfathiazole, rbc. permeability, 317.
Remington, J. W., Collings, W. D., Hays, H. W., and Swingle, W. W. Renin, assay, 470.
Reynolds, S. R. M., Kaminester, S., and Schloss, S. Menstruation, estrogen, 749.
Rice, L. 750.
Richardson, E. H., Jr. Allergy, auto-antigens, 787.
Riddle, J. W., Spies, T. D., and Hudson, N. P. Resistance, deficiency disease, 361.
Riley, R. F., Krause, R. F., Steadman, L. T., Hunter, F. E., and Hodge, H. C. Barbiturates, color reaction, 424.
Rivers, T. M. 669
Rocha E Silva, M. Histamine in skin, 586.
Rogers, R. E., Hirschmann, D. J., and Humfeld, H. *Trichophyton interdigitale,* growth, 729.
Rolf, D. 433.
Rosen, S. 449.
Ross, B. D. Calcification, 531.
Rothman, S. Tyrosine oxidation, ascorbic acid, 52.
Rovenstine, E. A. 415; 785.
Rubinstein, H. S., and Solomon, M. L. Testosterone, body growth, 745.
Russell, D. 62.
Rulon, O. Development of sand dollar, NaCNS, 23.

Salle, A. J., Schechmeister, I. L., and McOmie, W. A. Dyes, germicidal, 614.
Salmon, U. J., and Geist, S. H. Pregneninolone, reactions, 522; α-Estradiol, buccal absorption, 766.
Salmon, W. D., and Engel, R. W. Pantothenic acid, adrenal hemorrhage, 621.

Salzburg, P., and Kubicek, W. G. Vascular obstruction, 831.
Sampson, W. L. 309.
Sanders, M., and Molloy, E. Encephalomyelitis, equine, cultivation, 327.
Sapirstein, L. A. 573.
Sapirstein, L., Pollock, W., and Krichesky, B. Uterus extr., toxicity, 431.
Saunders, F. 750.
Schaffer, N. K., and Stander, H. J. Uric acid in eclampsia, 180.
Schattenberg, H. J. *B. violaceous*, 829.
Schloss, S. 749.
Schechmeister, I. L. 614.
Schiffrin, M. J. Ulcer, jejunal, 592.
Schmidt, L. H., Claugus, C. E., and Starks, E. Sulfonamides, pneumococci, 256.
Schwartz, L. 317.
Scott, G. H. Muscle Ca and Mg, electron microscope, 30.
Scott, K. G. 652.
Seegal, B. C., and Loeb, E. N. Fetal development, anti-placenta serum, 248.
Segal, A. L. 20.
Seifter, S. 130.
Selye, H., and Bassett, L. Desoxycorticosterone, testosterone, H_2O, Cl metabolism, 272.
Shaffer, M. F. 259.
Shamleffer, V. 31.
Sharpless, G. R. Choline, gastric epithelial hyperplasia, 487.
Shaw, J. H. 482.
Shelesnyak, M. C., Rosen, S., and Zacharias, L. R. Sexual function, sphenopalatine ganglia, 449.
Shepherd, M. L. 153.
Sherman, H. C. 589.
Sherrill, J. W. 437.
Shettles, L. B. Spermatozoa quiescence, CO_2, 318.
Shimkin, M. B., and Grady, H. G. Cancer, mammary, stilbestrol, 246.
Shohl, A. T., Blackfan, K. D., and Diamond, L. K. Cell, volume detn., 383.
Sia, R. H. P. 87.
Sigurdsson, B. Cells, antigenic properties, 237.
Silvette, H. Post.-pituitary, excretion, 599.
Simpson, M. E. 627.
Slaughter, D., and Lackey, R. W. Serum choline-esterase, 8.
Smadel, J. E. 669.
Smith, A. H. 630.
Smith, E., and Penrod, K. E. CO poisoning, sugar tolerance, insulin, 222.
Smith, M. G., and Reames, H. R. Encephalitis viruses, relation, 838.
Smith, W. W. Agglutinins, anti-bacterial, 726.
Snapp, E. 174.
Snyder, J. C., and Mueller, J. H. *C. diphtheriae*, nutrition, 243.
Solmssen, U. V. 407.
Solomon, M. L. 745.
Somogyi, M. Hypoglycemia, ketonemia, 644; and Kirstein, M. B. Fever, ketonemia, 640.
Souders, H. J. 153.
Spealman, C. R. Atrium preparation, pace maker, 189.
Spiegel, E. A. 418.
Spies, T. D. 361, 441.
Sprinz, H. 15.
Stacy, J. R. 784.
Stander, H. J. 180.
Stare, F. J. 525, 529.
Starks, E. 256.
Steadman, L. T. 424.
Steggerda, F. R., and Ponder, E. Nervous system, skin potentials, 617.
Steiner, A. 175.
Stekol, J. A. Dibenzyl disulfide to hippuric acid, 693.
Stephens, D. J., and Belasco, I. J. Thyroid respiration, undernutrition, 706.
Stevens, H. 798.
Steyermark, A. 407.
Stimpert, F. D. 665.
Stokes, M. Thyroid localization, 681.
Stone, C. P., Ferguson, L. W., and Wright, C. Ejaculatory quiescence, 120.
Stone, L. S., and Dinnean, F. L. Lens, origin, 183; and Ellison, F. S. Eye, heterotransplantation, 181.
Strong, L. C. 287.
Swank, R. L. 704.
Sweet, J. E., and Hoskins, W. H. Androgen, hibernating gland, 60.
Swingle, W. W. 470.

Taffel, M., and Harvey, S. C. Sulfonamides, wound healing, 647.
Takacs, W. S. 364.
Taliaferro, I. 479.
Tashiro, S., Badger, E., and Younker, W. Chromodacryorrhea, 377.
Teague, D. M. 153.
Telford, I. R., Emerson, G. A., and Evans, H. M. Muscle lesions in E-low rats, 135.
Templeton, R. D., Galapeaux, E. A., and Adler, H. F. Colon, morphine effect, 98.
Thienes, C. H. 109, 682.
Thorp, W. T. S. 50.
Tipton, S. R. Adrenalectomy, tissue respiration, 596.
Tobie, J. E. *Endamoeba histolytica*, pathogenicity, 691.
Tocantins, L. M. Absorption from bone marrow, 292; and O'Neill, J. F. Bone marrow, infusion, 782.
Toomey, J. A., and Takacs, W. S. Poliomyelitis, transmission, 364.
Torrance, C. C. Skin lesions, oxidation potentials, 741.
Toscani, V. 394, 397.
Travell, J. Nicotine absorption, pH, 552.
Tuttle, L. W. 652.
Tyler, D. B. Insulin shock, pentobarbital, brain damage, 117, 682.

Unna, K., and Greslin, J. Pantothenic acid, toxicity, 311; and Sampson, W. L. Pantothenic acid, achromotrichia, 309.

Vance, R. W., Snapp, E., and Ivy, A. C. Glycogen, liver, bile extract, 174.
Van der Scheer, J. 323; and Wyckoff, R. W. G. Sera, antitoxic, electrophoresis, 634.
VanVoorhis, S. N. 70.
Vargas, L. 788.
Virtue, R. W. 813.

Wagreich, H., Abrams, A., and Harrow, B. Benzoic acid detoxication, glucuronic acid, 46.
Waksman, D. A., and Woodruff, H. B. Actinomyces, bactericidal subst., production, 609.
Ward, S. M. 669.
Wasserman, P., and Alberts, E. Complement of newborn, 563.
Weatherby, J. H. Nicotine depressant, ciliated epithelium, 284.
Webster, B., and Hoskins, W. Androgen therapy, growth, 72.
Webster, L. T. Encephalitis viruses, immunology, 499.
Wedum, A. G. Sulfonamide azoproteins, immunology, 218.
Weech, A. A. Photometer, micro., 559; 858.
Wegner, M. I., Booth, A. N., Elvehjem, C. A., and Hart, E. B. Vitamin B, rumen synthesis, 769.

Weiss, S. 306.
Wells, J. A., Burrill, M. W., and Greene, R. R. Testosterone, by mouth, 452.
Wesson, L. G. 298.
White, H. L. 623; and Rolf, D. Iodine detn. plasma, urine, 433.
Wick, A. N., Sherrill, J. W., and MacKay, E. M. Ketosis, fasting, 437.
Wiggers, C. J. 353, 355.
Wigodsky, H. S. 313, 373.
Wilbur, K. M. Colchicine, viscosity of egg, 696.
Williams, H. H. 153; Erickson, B. N., Bernstein, S. S., and Macy, I. G. Blood phospholipid partition, pernicious anemia, 151.
Wilson, P. W. 721.
Wirts, C. W., Jr. 253.
Witschi, E., and Fugo, N. W. Sex characters, hormone response, 10.
Wolbach, S. B. 298.
Womack, N. A., and Bricker, E. M. Gall bladder, pathology, 710.
Woolley, D. W., and McCarter, J. R. Antihemorrhagic compd., Johne's bacillus, growth factor, 357.
Woolley, G., Fekete, E., and Little, C. C. Cancer, mammary, ovariectomy, 796.
Woo, T. T. 90.
Wood, W. B., Jr. Sulfapyridine, pneumonia lesions, 348.
Woodruff, H. B. 609.
Wong, S. C. *Corynebacterium diphtheriae* immunology, 850; and Ouyang, G. Tuberculin, stability, 83; and Ouyang, G. Tuberculin, 845.
Wortis, J. 468.
Wright, C. 120.
Wright, F. H. Encephalitis, resistance and foster-nursing, 871.
Wright, W. T. 441.
Wyckoff, R. W. G. 323, 634.

Yen, C. H., and Chen, C. Y. *B. typhosus*, excretion, VI-phage, 78.
Yolton, N., and Rea, C. Androgen, estrogen excretion, mammary cancer, 54.
Youmans, W. B. Intestinal denervation, motor responses, 420.
Young, A. F. 353.
Younker, W. 377.

Zacharias, L. R. 449.
Zander, D. 303.
Zapata, E. M. 701.
Zschiesche, E., and Mitchell, H. K. Pantothenic acid, 565.
Zener, F. B. 667.
Zia, S. H. 76; 823.

SUBJECT INDEX

(*The numerals indicate the page.*)

Achromotrichia, 773.
Actinomyces, bactericidal subst., production, 609.
Adrenal, fetal androgens, 502.
    necrosis, pantothenic acid, 482.
Adrenalectomy, cbhy. stores, NaCl, 488.
    lymphoid tissues, 267.
    tissue respiration, 596.
Agglutinins, anti-bacterial, 726.
Allergy, auto-antigens, 787.
Amino acids, absorption, 475.
Anaphylaxis, histaminase, 606.
Anesthesia, spinal, venous pressure, 415.
Anoxia, economy effort index, 353.
Anti-gonadotropic subst., pregnant mare serum, 506.
Antihemorrhagic compd., 55, 407, 412.
    growth factor for Johne's bacillus, 357.
Antigen purification, grass pollen, 460.
    ragweed pollen, 637.
    extract, sensitization, 798.
Arginase, liver, fasting, 25.
Atherosclerosis, high protein diet, 543.

Bacillus, Johne's, growth factor, 357.
    typhoid, typing, 400.
    typhosus, excretion VI-phage, 78.
    violaceous, 829.
Bacteria, O_2 needs, 454.
Barbiturates, color reaction, 424.
Bee, carbohydrates, 754.
Benzoic acid detoxication, glucuronic acid, 46.
Bile, bile salt effect, 313.
Blood, alimentary absorption, 569.
    ascorbic acid, pellagra, 441.
    cell volume detn., 383.
    clot, phospholipids, 579.
    coagulation, collodion vessels, 122.
    elements, cerebroside, neutral fats, 153.
    erythrocytes, gastric juice, 373.
        hemolysis, 744.
    leucocytes diurnal levels, 863.
    phospholipid, pernicious anemia, 151.
    plasma K, epinephrine, liver, 266.
    polycythemia, cobalt, 671.
    sugar, temperature, 392.
    sera, antitoxic, electrophoresis, 634.
        bilirubin, 253.
        choline-esterase, 8.
        electrolytes, hyperpyrexia, 427.
        rickettsial, refined, 323.
Body temperature, drug toxicity, 682.
Bone conduction, sensitivity, 662.
    marrow, absorption from, 292.
        infusion, 782.
Brown fat extr., metabolism, 37.
Brucella abortus, immune response, 801.

Calcification, 531.
Canavanine, prepn., structure, 224.
Cancer, mammary, milk, 805.
    ovariectomy, 796.
    stilbestrol, 246.
    thyrotropic hormone, 820.
Caproic acids, metabolism, 96.
Carcinoma, Brown-Pearce, antibody, 517.
Carrion's disease, 546.
    cultivation, 402.
Caudate, labyrinthine disregard, 473.
Cells, antigenic properties, 237.
Chick diet, charcoal, 303.
Chloroform poisoning, thyroxin, 201.
Choline, gastric epithelial hyperplasia, 487.
Chromodacryorrhea, 377.
    cyclopropane, 785.
Cinchophen, detn. in bile, 143.
    excretion in bile, 853.
Circling, brain stem lesions, 389.
Circulation, peripheral, fatigue, 685.
C. diphtheriae, nutrition, 243.
    oleic acid, 244.
Clostridium tetani, cultivation, 826.
CO poisoning, sugar tolerance, insulin, 222.
Colchicine, viscosity of egg, 696.
Colon, morphine effect, 98.
Complement, fat solvents, 130.
    newborn, 563.
    thermostability, 115.
Conditioned reactions, cortical lesions, 418.
Corynebacterium diphtheriae immunology, 850.
Coumingine HCl, susceptibility, 209.
Cymarin, susceptibility, 209.

Dental plaque metabolism, 104.
Dermatitis in chickens, 495.
Development, embryonic, 689.
    sand dollar, NaCNS, 23.
Dibenzyl disulfide to hippuric acid, 693.
Digoxin, pharmacology, 323.
Diphtheria toxin, susceptibility, 165.
Dyes, germicidal, 614.

Ejaculatory quiescence, 120.
Electrocardiogram, O_2 and CO_2 inhalation, 175.
Embryo, chick, cbhy. metabolism, 278.
    development, 351.
Encephalitis, resistance and foster-nursing, 871.
    immunology, 499.
    viruses, relation, 838.
Encephalogram, electrode, 301.
Encephalomyelitis, equine, cultivation, 327.
Endamoeba histolytica, pathogenicity, 691.
Estrous cycle, nephrectomy, 226.
Ethylaminobenzoate, anesthetic, 337.
Eye, heterotransplantation, 181.

Fetal development, anti-placenta serum, 248.
Fever, ketonemia, 640.
Fluorescence, protein, 792.

Gall bladder, pathology, 710.
Gastric adenoma, *Dysticercus fasciolaris*, 673.
Glutathione, protein oxidation, 529.
Glycogen, liver, bile extract, 174.
Grass culture, soilless, 62.

Heart, action current, KCl, 263.
    atrium preparation, pace maker, 189.
    fibrillation, recovery, 355.
Histamine in skin, 586.
Hormone, adrenal cortex, muscle efficiency, 602.
    Androgen, estrogen excretion, mammary cancer, 54.
        hibernating gland, 60.
        response, 780.
        therapy, growth, 72.
        uterine wt., 34.
    Desoxycorticosterone, H_2O, Cl., 272.
    Epinephrine, respiration, B.P., 776.
    α-Estradiol, buccal absorption, 766.
    Estrogens, comparison, 594.
        femur strength, 230.
        fibroids, 788.
        implantation, 659.
    Insulin, crayfish kidney, 873.
        hypoglycemia, cerebral blood flow, 468.
        shock, pentobarbital, brain damage, 117.
    Pitressin, activation, 573.
    Pituitary melanophore hormone, chick embryo, 810.

   ant., follicular hormone, 627.
   thyrotropic, assay, 772.
   post., excretion, 599.
   water retention, 20.
  Pregneninolone, reactions, 522.
  Progesterone, uterus, 270.
  Prolactin, water drive factor, 335.
  Sex, excretion in mammary cancer, 54.
  Testosterone, anal fin regeneration, 484.
   body growth, 745.
   by mouth, 452.
   metabolism, 739.
   H_2O, Cl., 272.
Hypertension, antipressor effect of blood, 185.
  blood flow, 127.
Hyperthyroidism, alkali reserve, acid excretion, 196.
Hypoglycemia, ketonemia, 644.

Influenza antigen, antibody, 439.
  differentiation, 861.
Intestinal denervation, motor responses, 420.
  loops, preparation, 813.
Iodine, detn., plasma, urine, 433.
Isoagglutinins, atypical, 346.

Jaundice test, 405.

Ketosis, fasting, 437.
Kidney, diodrast, inulin clearances, 623.
  hypertrophy, testosterone, 216.

Lactic acid, mouth, 464.
Lanatoside C, pharmacology, 323.
Lens, origin, 183.
Leucine and isoleucine, detn., 289.
Leukemia, cells, irradiation, 131.
  methylcholanthrene, 287.
  sp. subst. from urine, 387.
Liver, kidney damage, Na sulfamethylthiazole, 447.
Lymphogranuloma venereum, Frei test, 259.

Mamma, hypophysectomy, 835.
Melanophore hormone, 493.
  steroids, 571.
Meningococcus, egg yolk, 848.
Menstruation, estrogen, 749.
Metabolism, fasting, 630.
Methylation, choline, creatine, 657.
Muscle Ca and Mg, electron microscope, 30.
  K uptake, 817.
  lesions in E-low rats, 135.
  single response, 650.
Myopathy prevention, α-tocopherol, 457.

N. gonorrhoeae, culture, 381.
Nerves, cross-excitation, veratrine, 870.
Nervous system, skin potentials, 617.
Nicotine absorption, pH, 552.
  depressant, ciliated epithelium, 284.

Oncometry, 5.
Ouabain, susceptibility, 209.

Pancreas necrosis, Ca, 803.
Pellagra, blood ascorbic acid, 441.
Peritonitis, prevention, 660.
Pernicious anemia-like syndrome, 794.
Phenothiazine, oxidation, 632.
Phosphorus, determination, 559.
  distribution in bone, teeth, 70.
  effect on epiphysis, 539.
  radio, retention, 652.
Photometer, micro, 858.
Pig lameness, Mn, 50.
Plasmodium cathemerium, sporozoites, 477.
Pneumococcus, pptn. of specific substances, 93.
Pneumonia, virus, 677.
Poliomyelitis, susceptibility, 232.
  transmissions, 364; 665.
  virus propagation, 124.
Pregnancy, I therapy and B.M.R., 667.
  isoimmunization, 343.
Procaine and atropine, 109.
Prospermin, pituitary, 497.

Radioactive ion distribution, 856.
Relapsing fever spirochetes, cultivation, 375.
Renin, assay, 470.
Reproduction and vitamin B, 625.
Resistance, deficiency disease, 361.
Respiratory activity of cells, 721.
  center localization, 686.
Rhenium, physiology, 576.
Rickets, fluorine, 843.
  low Ca, 298.
Roentgen-pigmentation, 148.

Scrotum, development, castration, 867.
Sex characters, hormone response, 10.
Sexual function, sphenopalatine ganglia, 449.
Skin lesions, oxidation potentials, 741.
Spermatozoa quiescence, CO_2, 318.
Streptococci, gramicidin, 462.
  hemolytic, 509.
  virulence tests, 87.
Strontium in milk, 762.
Sulfonamide azoproteins, immunology, 218.
  b. pyocyaneus, 709.
  Brucella, 512.
  dysentery respiration, 750.
  enteritidis, 31.
  excretion, 734.
  liver and kidney damage, 447.
  Na salts, toxicity, 15.
  pneumococci, 256.
  pneumonia lesions, 348.
  rbc., permeability, 317.
  Shigella paradysenteriae, 111.
  tissue response, 536.
  tuberculosis, 556.
  typhoid-dysentery group, 169.
  wound healing, 647.

Thymus, pregnancy involution, 315.
Thyroid localization, 681.
  respiration, undernutrition, 706.
  tissue histamine, 688.
Tissue metabolism, detn., 137.
  drugs, 140.
Trichinella spiralis, incidence, 701.
Trichomonas vaginalis, cell size, pH, 220, 567.
Trichophyton interdigitale, growth, 729.
Triphenyl phosphite, toxicity, 306.
Tryptase, heparin inhibition, 43.
Tuberculin, 845.
  stability, 83.
Tumor incidence, proline, 601.
  tissue oxidation, 525.
Typhus culture, 76.
  rickettsia, 823.
Tyrosine oxidation, ascorbic acid, 52.

Ulcer, cinchophen, 66.
  gastric, diet and cinchophen, 190.
  jejunal, 592.
Uterus extr., toxicity, 431.
  toxicity after estrogen, 214.
Uric acid in eclampsia, 180.

Vaccinia, biotin, 669.
Valine, detn., 289.
Valve, mercury, 207.
Vascular obstruction, 831.
Virus, influenza, electrophoresis, 679.
  influenza-like, 162.
  Mexican typhus, protection, 40.
  psittacosis, thiamin deficiency, 704.
  rabbit papilloma, inactivation, 713.
  rabies, properties, 332.

    Rous sarcoma, protection against, 367.
    Theiler's, immunology, 339.
Vitamin A of tissues, 589.
    Ascorbic acid, colon, 479.
        creatin metabolism, 90.
        hypophysectomy, 784.
        pellagra, 441.
        tyrosine oxidation, 52.
    B, rumen synthesis, 769.
    B$_1$ deficiency, insulin, 759.
        excretion, 1.
        infant requirement, 765.
    B$_6$ deficiency, cure, 717.
    Biotin, formation in rumen, 296.
        vaccinia, 669.
    C, creatine excretion, shivering, iodoacetate, epinephrine, 397.
        metabolism, 90.
        excretion, liver injury, 394.
    E deficiency, muscle lesions, 135.
        E-low rats, 159.
    Flavin-dinucleotide, arbacia ova, 719.
    Nicotinic acid, growth, 598.
    Pantothenic acid, 565.
        achromotrichia, 309.
        adrenal hemorrhage, 621.
        toxicity, 311.
    Tocopherol, daily need, 715.
        growth, 157.
        muscle lesions, 583.

Zinc deficiency, 282.